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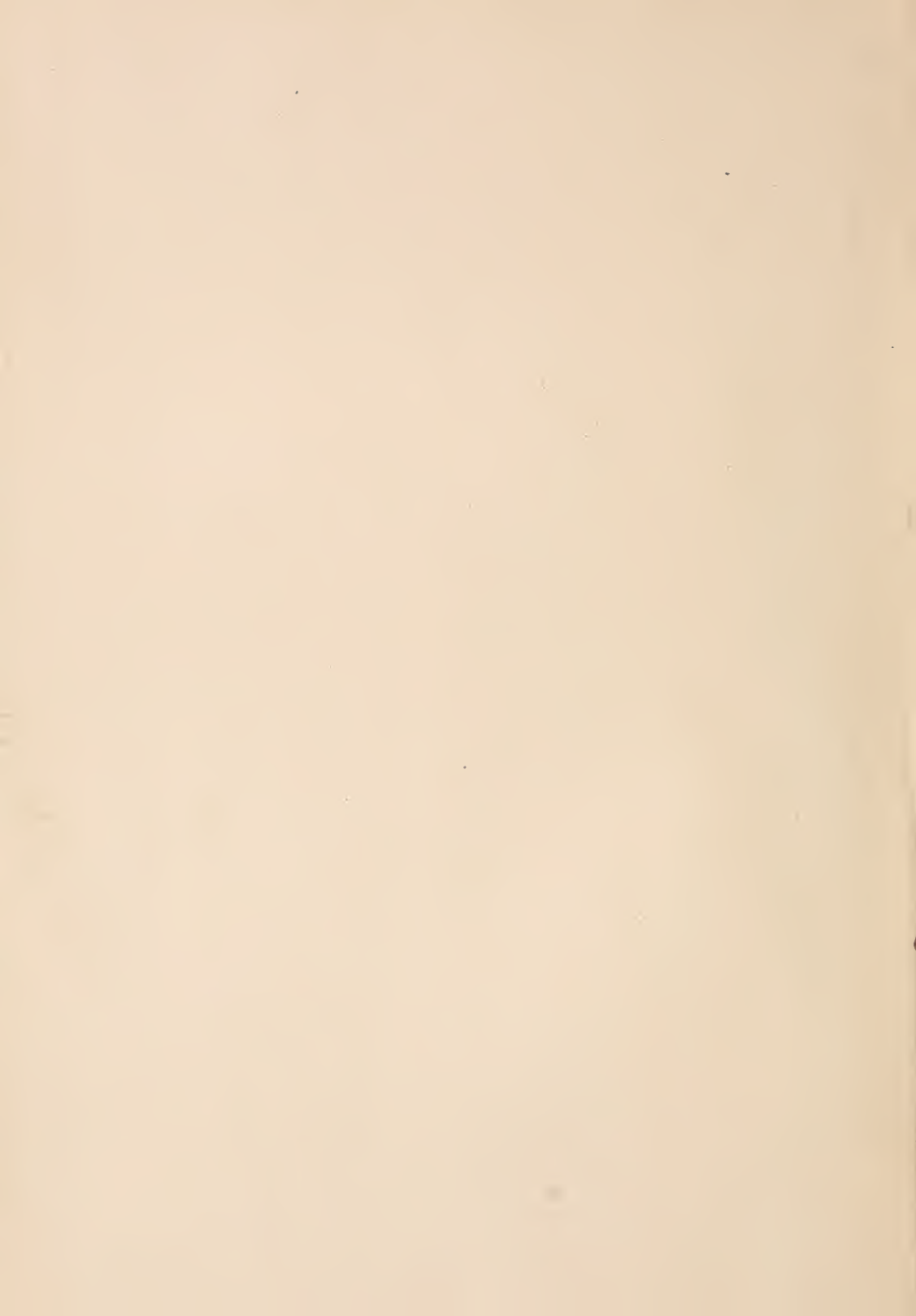
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THE  
JOURNAL OF INFECTIOUS DISEASES



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# *The* Journal of Infectious Diseases

Founded by the Memorial Institute for Infectious Diseases

EDITED BY

LUDVIG HEKTOEN AND EDWIN O. JORDAN

IN CONJUNCTION WITH

FRANK BILLINGS      F. G. NOVY  
W. T. SEDGWICK

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*Supplement No. 3, May, 1907*

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With Table of Contents and Index for Supplement Nos. 1, 2, and 3

*Some of the Papers presented to the Laboratory Section  
of the American Public Health Association  
at the Mexico City Meeting,  
Dec. 3, 1906*

Chicago, 1907

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# *The* Journal of Infectious Diseases

FOUNDED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

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*Supplement No. 1, May, 1905*

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## REPORT OF COMMITTEE ON STANDARD METHODS OF WATER ANALYSIS TO THE LABORATORY SECTION OF THE AMERICAN PUBLIC HEALTH ASSOCIATION.

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## LETTER OF TRANSMITTAL.

NEW YORK, December 19, 1904.

*To the Chairman and Members of the Laboratory Section of the American Public Health Association.*

GENTLEMEN: The final report of your Committee on Standard Methods of Water Analysis is submitted herewith.

It is now more than ten years since there arose in North America a movement for securing the adoption of more uniform and efficient methods for water analysis, particularly of bacteriological methods. Earlier accomplishments<sup>1</sup> related largely to uniform methods of recording chemical results. The late Dr. Wyatt Johnston of Montreal was the first one to call the attention of the Association to this line of work, and at the Montreal meeting in 1894 the first step<sup>2</sup> in this direction was taken. By invitation of a subcommittee of the Committee on Pollution of Water Supplies, a convention<sup>3</sup> of American bacteriologists assembled in New York in June, 1895, and appointed a committee to draw up procedures for the study of bacteria in a uniform manner and with special reference to the differentiation of species. This committee submitted a report<sup>4</sup> at the Philadelphia meeting of the Association in 1897. It was published early in 1898 and has been widely used in various laboratories in

this country. The demand for copies of it still continues, although for some time it has been out of print.

At the Minneapolis meeting in 1899 the present committee was appointed with a view to extending the standard procedures to include not only the determinations of species of bacteria, but all the other lines of investigation involved in the analysis of water.

The committee undertook first to ascertain the views of the analysts of America regarding not only the bacteriological, but also the chemical, physical and microscopical examinations of water. Circular letters were sent to all the principal laboratories, and much coöperative work was done in connection with the differentiation of species of bacteria.

Progress reports were made at meetings held at Indianapolis, Buffalo and New Orleans in 1900, 1901 and 1902, the two latter being published in the *Proceedings of the Association* for those years, and the former in *Science*.<sup>5</sup>

In 1901 this committee was instructed to revise the 1897 report of the Bacteriological Committee in order to exclude from it those features not found to have been of general service and to include such new matter as later developments had justified.

In 1902 the committee suffered a severe loss through the death of one of its most valued members, the late Dr. Wyatt Johnston of Montreal, who is credited by all with the initiation of this movement. Dr. Adolph Gehrmann of Chicago resigned from the committee in that year. At the New Orleans meeting the full membership of the committee was restored by the appointment of Mr. R. S. Weston of Boston and Mr. J. W. Ellms of Cincinnati.

The past decade has been a transitional period for water analysis. As this field of sanitary investigation has been extended to cover in a more thorough manner the conditions existing throughout this country, it has been found that the methods applicable to the fairly clear waters of the Atlantic seaboard and to many European waters have not been adequate for the analysis of the muddy waters of the South and West. Conversely, new methods used in certain sections of the country are for similar reasons not always applicable to the conditions existing where the earlier studies were made.

The purification of water supplies has received a great impetus during the past decade, and the water analyst is more and more being placed in charge of the operation of large public filter plants. In work of this character, especially when muddy waters are being treated, he thus uses some methods of analysis which are not generally required for sanitary work; while, on the other hand, he omits with propriety various tests which form an essential part of the customary sanitary analysis.

The presence of objectionable amounts of iron in various ground water supplies and the introduction of special processes for its removal have in some instances severely taxed the resources of the water analyst, and necessitated modifications of old methods.

Methods for determining lead and copper are being used with more frequency than was the case in earlier years, when less attention was given to all the aspects of public health.

Treatment of sewage, to prevent gross nuisances and to prevent the pollution and infection of streams, has recently received a marked stimulus through the development of the so-called rapid biological filters, the septic

tank treatment, etc. Special tests and analyses have been required in connection with these lines of work—notably those for the determination of the putrescibility of the effluents of sewage works. The study of the longevity of disease germs in connection with the question of infection of water supplies drawn from streams at various distances below the discharge of outfall sewers has brought into prominence new lines of bacteriological investigation.

Enough has been said in outline of the new and varied requirements made of the water analyst to show the need of a broad and substantial basis for his methods and for his work under present conditions. Some of the older methods, used for the study of the general sanitary quality of unfiltered water supplies, are becoming less and less important, while the newer ones, used in the operation of purification plants, are becoming of more value. This is in keeping with the modern tendency of the analyst to become more and more an important factor in connection with the operation of plants for the purification of water and sewage. Indeed, unpurified sources of water supply are becoming fewer and fewer, as hygienic demands are being met by the rapid introduction of purification works, as evidenced by the best practice both in Europe and in America.

The methods of analysis presented in this report as “standard methods” are believed to represent the best current practice of American water analysts, and to be generally applicable in connection with the ordinary problems of water purification, sewage disposal and sanitary investigations. Analysts working on widely different problems manifestly cannot use methods which are identical, and special problems obviously require the methods best adapted to them; but, while recognizing these facts, it yet remains true that sound progress in analytical work will advance in proportion to the general adoption of methods which are reliable, uniform and adequate.

It is said by some that standard methods within the field of applied science tend to stifle investigation, and that they retard true progress. If such standards are used in the proper spirit this ought not to be so. The committee strongly desires that every effort shall be continued to improve the technique of water analysis, and especially to compare current methods with those herein recommended, where different, so that the results obtained may become still more accurate and reliable than they are at present.

In Table No. 1 are given the more essential determinations which, in the opinion of the committee, should be applied to each of the principal lines of analytical work in connection with the ordinary problems of water supply and sewage disposal. It is realized that some of the older laboratories are hardly in a position at the present time to follow out these suggestions in a literal manner, although on new work it is believed that they could follow them to advantage.

Some of these suggestions may seem radical, and in special instances they may be indeed inexpedient, as already mentioned, but on the whole it is believed that they indicate the lines along which the water analyst may direct his efforts to best advantage and with the feeling that he is obtaining all the data necessary, while doing little or nothing that is needless.

It will be noted that the bacteriological determinations, including the tests for *Bacillus coli*, are given much more prominence than was for-



merly the case, and that less attention is given to the organic matter as determined by chemical analysis. This is because of the inability of the chemical methods to separate that portion of the organic matter which is of no sanitary significance from that which is associated with pollution or infection.

The most substantial steps in advance relate to improvements in the physical and chemical methods required in connection with the operation of plants for the improvement and purification of water supplies.

Detailed descriptions of the various methods recommended are given in concise form, covering the essential features of each determination. It is assumed that those using these directions are thoroughly grounded in the fundamental principles of chemistry and biology, and that they are also familiar with the leading literature upon the subject. So many satisfactory textbooks upon chemical analysis in general and on water analysis in particular are in existence that it is unnecessary to give a complete detailed description of all procedures; but it is fully recognized that in many cases the adherence to certain details is an essential matter, and hence for the newer methods they are incorporated in this report.

Some of the methods described are known in different parts of America by different proper names, hence it has been the endeavor of the committee to describe them with sufficient clearness to make plain what procedures are meant without reference to the name of the author, but to give due credit for the method by referring to his published work in the bibliography at the end of the report.

The bibliography is by no means a full list of important works on water analysis. It is simply a list of references to the works most consulted in America, arranged for the purpose of assisting the reader in getting in touch with the general aspects of a method, including its history and application, together with full technical details of the procedure as now practiced.

No attempt is made to report upon the interpretation of the results of water analyses, or upon the classification of bacteria, as these subjects are receiving the attention of other committees of the Association,

This report does not deal with any of the numerous phases of applied bacteriology in the domains of medicine or industrial science. It is hoped, however, that workers in these fields may find useful portions dealing with the preparation of media, and that published descriptions of bacteria associated with disease or with various industrial processes will be made to conform with the procedures herein recommended.

Very respectfully,

(Signed) GEORGE W. FULLER, *Chairman.*  
GEORGE C. WHIPPLE, *Secretary.*  
H. W. CLARK.  
EDWIN O. JORDAN.  
H. L. RUSSELL.  
J. W. ELLMS.  
ROBERT SPURR WESTON.

TABLE I.\*  
PROPOSED TESTS FOR OBTAINING ESSENTIAL DATA WITH VARIOUS TYPES OF WATERS.

	SURFACE WATERS				GROUND WATERS	WATERS BEFORE AND AFTER TREATMENT BY				SEWAGES	
	Clear	Col-ored	Turbid	Highly Pol-luted		Sand Filters	Mechan-ical Filters	Soft-en-ing Plants	Iron Remov-ing Plants	Before Treat-ment	After Treat-ment
Physical examination <div><div><div><div>Temperature</div><div>Turbidity</div><div>Color</div><div>Tint and shade</div><div>Odor</div></div><div>Oxygen consumed</div><div>Nitro-gen as <div><div>Organic nitrogen</div><div>Albuminoid ammonia</div><div>Free ammonia</div><div>Nitrites</div><div>Nitrates</div><div>Total</div></div></div></div><div><div>Residue on evaporation</div><div>Suspended</div><div>Dissolved</div><div>Loss on ignition</div><div>Fixed residue</div></div></div>	++ ++ 0 0	++ ++ ++ 0	++ ++ ++ 0	++ ++ 0 0	++ ++ 0 0	++ ++ 0 0	++ ++ 0 0	++ ++ 0 0	++ ++ 0 0	++ ++ 0 0	
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\* For observations and comments see next page.

## OBSERVATIONS ON TABLE 1.

Plus (+) sign indicates that the test is advised for each type of water under which it appears; while a zero (0) indicates that the test is not advised.

This table indicates the general views of the committee, and is not intended to limit the use of other tests, either specially or regularly, where the local conditions show such supplementary tests to be desirable.

In some instances the tests need not be made on all samples, such, for example, as in the case of dissolved oxygen, and putrescibility of sewage before treatment. The plus signs are here used to show that the information conveyed by these tests should be known to the analyst; and that it is unsafe to assume that all sewages are putrescible or lacking in dissolved oxygen during all hours of the day. Turbidity, free carbonic acid, alkalinity and sulphate contents at intervals are also valuable in sewage work.

Free mineral acids and sulphates are important data for surface waters in the coal regions.

Tests for lead, copper, tin, and zinc are frequently called for in studying the effect of waters upon service pipes.

Whenever chemicals are applied to water for any purpose, the results of analyses should record the disposition of the applied chemicals. Water treated by mechanical filters should be tested frequently for acidity, that is, undecomposed coagulant. Softened waters should be tested for caustic alkalinity in a thorough manner as detailed under hardness.

## INTRODUCTION TO THE 1897 REPORT OF THE BACTERIOLOGICAL COMMITTEE.

As explained by Dr. Smart in the preface to this report, a convention of bacteriologists from the United States and Canada assembled in the city of New York, on June 21 and 22, 1895, in response to the invitation of a sub-committee of the Committee on the Pollution of Water Supplies of the American Public Health Association. The proceedings of this convention, including the papers read and their discussion, were published in the *Journal of the American Public Health Association*, October, 1895. These papers and discussions related mainly to technical procedures to be followed in the systematic study of bacteria, with especial reference to their description and identification. There was general agreement of opinion as to the importance of securing greater precision and uniformity in the methods of studying and describing bacterial species. A committee of members of the convention was therefore appointed to prepare a report, to be presented to the Water Committee of the American Public Health Association, this report to contain recommendations concerning bacteriological methods based partly upon the deliberations of the convention and partly upon a wider study of the subject. The members selected for this committee were Drs. J. George Adami, William T. Sedgwick, George W. Fuller, Charles Smart, Alexander C. Abbott, T. M. Cheesman, Theobald Smith, and William H. Welch.

A first draft of a report was drawn up by Dr. Adami and submitted to the members of the committee, who made various suggestions. The final preparation of the report was undertaken by Dr. T. M. Cheesman, Instructor in Bacteriology in the College of Physicians and Surgeons, Columbia University, New York. The following statement by Dr. Adami well expresses the aims and manner of preparation of the report:

Naturally, with a committee, the members of which are so widely scattered, it has been found impossible to hold frequent meetings, but at these meetings the members have found themselves singularly in accord upon everything

relating to the main points at issue. Naturally, also, correspondence and the circulation of the report in its various stages have not been found-entirely satisfactory in eliciting the opinions of every member upon matters of detail. But all these means accomplished much, and it was eventually found possible to place the final drafting of the recommendations in the hands of one member. We cannot sufficiently express our indebtedness to Dr. Cheesman for the amount of time, and indeed of independent work which he has devoted to this task.

The recommendations thus do not indicate the previous procedure in all details of any single member of the committee, but are a concord of what has appeared to be the best in the methods and technique of all the members and of bacteriologists generally. To have indicated in the following pages wherein any single member found himself unable to accept in its entirety any one of the many recommendations would have counteracted our main object, that, mainly, of inducing uniformity and precision in procedure in the study and descriptions of species. Each member, therefore, to attain this object has voluntarily refrained from demanding that one or other method, to which from long employment he has become firmly attached, should be inserted in these pages. The committee freely admits that there may be other and better methods than those here detailed. It has, on the other hand, striven to recommend what in the present state of our knowledge would seem to be the best and most likely to gain acceptance. It does not demand of bacteriologists in general — it does not promise for its own members in particular — that these and only these methods shall be employed. It does but ask that *where new species are being studied for publication* the procedure here recommended be given a trial, and that, for the direction of other workers, where it has been employed a note be given to that effect, e. g., "cultures in broth (Method B. C.) presented the following characters:" — or, "save where otherwise indicated, the B. C. methods have been used."

In short, the committee recognizes fully that these recommendations must of necessity be provisional. It publishes them in the hope that by this act it will direct attention to the urgent need now existing for full and accurate descriptions of species of bacteria in which the items have been determined by methods common to the main body of workers, and as a consequence are capable of verification and control.

The report is not intended to be a complete treatise upon bacteriological technique. Its purpose is to make certain recommendations concerning methods to be pursued in the study of bacteria, with the view of securing greater uniformity and exactness in the determination and description of the characters of bacterial species. When one considers the difficulty, often the impossibility, of the identification of many bacterial species or varieties described in literature, in consequence of imperfections and carelessness in the determination and description of their characters, it is evident that the attainment of the purpose aimed at in this report is greatly to be desired.

The report deals especially with certain ordinary and fundamental procedures in bacteriological technique, and it does not attempt to cover fully the entire field. In a science so rapidly developing as bacteriology, it need scarcely be said that any attempt to present the best technical procedures can apply only to the existing state of the science, and that much will be added and much corrected in the near future. It is hoped that the recommendations in this report may prove useful to workers in bacteriology, and especially may lead to greater accuracy and fullness and uniformity in the determination and description of the characters of bacteria.

(Signed)      WILLIAM H. WELCH.



## PREFACE TO THE 1897 REPORT OF THE BACTERIOLOGICAL COMMITTEE.

At the meeting of the American Public Health Association in Montreal, Canada, in 1894, the committee on the Pollution of Water Supplies closed its report with the suggestion of a coöperative investigation into the bacteriology of water supplies as a means of bringing order out of the chaotic state of the literature of water bacteria, and of throwing light from the bacteriological side on questions of practical sanitation. This suggestion was approved by the Association and the Chairman of the Committee was authorized to build up a committee for collective bacteriological investigation. The bacteriologists promptly acceded to the proposition. They recognized that such an investigation would give an immense impetus to bacteriological work; that it would do much to clear away the confusion surrounding species, and to increase and systematize our knowledge; and that practical results might also be expected, particularly as regards the typhoid and colon bacilli, the unwholesomeness of water supplies and the means of lessening the prevalence of typhoid fever and diarrheal diseases. A sub-committee consisting of Professor J. George Adami, Dr. Wyatt Johnston, Mr. George W. Fuller and myself, appointed to determine the methods of laboratory procedure to be adopted by the committee in the practical work of the investigation, found it impossible to formulate a satisfactory scheme of work until certain questions, mostly relating to technique, had been discussed fully and settled in accordance with the most advanced knowledge of the subjects concerned. An effort to effect this by correspondence developed so much variation in the practice of the different laboratories that it became needful to call a convention for a thorough discussion of the points at issue. The convention was held in the Academy of Medicine, New York City, June 21 and 22, 1895. Most of the prominent bacteriologists of the United States and Canada were present, but although the members were informed beforehand of the subjects that were to be brought up for settlement, and although full discussion was given to each under the chairmanship of Professor Welch of Johns Hopkins University, many of the points presented so much difficulty that the whole series was referred to a committee, with the understanding that the convention would accept its decision.

This committee consisted of

- J. George Adami, McGill University, Chairman.
- A. C. Abbott, University of Pennsylvania.
- T. M. Cheesman, College Physicians and Surgeons, New York.
- George W. Fuller, Louisville Water Company.
- W. T. Sedgwick, State Board of Health, Massachusetts.
- Charles Smart, U. S. Army.
- Theobald Smith, Harvard University.
- W. H. Welch, Johns Hopkins University.

The committee met in New York City in February 1896 to digest its material and outline its report which was presented to the American Public Health Association at its meeting in Buffalo, New York, in September of that



year. The report was subsequently withdrawn for further criticism and amendment, and was finally submitted for publication at the meeting of the Association in Philadelphia, Pennsylvania, September, 1897.

(Signed) CHARLES SMART.

#### ACKNOWLEDGMENTS.

During the five years that the Committee on Standard Methods of Water Analysis has been in session, it has been in correspondence with most of the leading bacteriologists and sanitary chemists of America, and many of them have engaged in the practical coöperative work of comparing methods. To all of those who have thus aided in this work the Committee wishes to express its appreciation and thanks. The following is a list of those who deserve special recognition:

#### MEMBERS OF THE OLD COMMITTEE WHO HAVE GIVEN ADVICE IN CONNECTION WITH THE PRESENT REPORT.

Abbott, A. C.	- - - -	Philadelphia, Pa.
Adami, J. George	- - - -	Montreal, Que.
Sedgwick, William T.	- - - -	Boston, Mass.
Smith, Theobald	- - - -	Boston, Mass.
Welch, William H.	- - - -	Baltimore, Md.

#### LIST OF THOSE WHO HAVE ACTIVELY COÖPERATED WITH THE COMMITTEE IN THE PREPARATION OF THIS REPORT.

Copeland, W. R.	- - - -	Columbus, O.
Forbes, F. B.	- - - -	Boston, Mass.
Gage, S. DeM.	- - - -	Lawrence, Mass.
Harris, Norman MacL.	- - - -	Chicago, Ill.
Hazen, Allen	- - - -	New York, N. Y.
Hill, Hibbert W.	- - - -	Boston, Mass.
Johnson, George A.	- - - -	Columbus, O.
Kimberly, A. E.	- - - -	Columbus, O.
Kinnicutt, L. P.	- - - -	Worcester, Mass.
Moore, V. A.	- - - -	Ithaca, N. Y.
Park, William H.	- - - -	New York, N. Y.
Phelps, Earl B.	- - - -	Boston, Mass.
Prescott, S. C.	- - - -	Boston, Mass.
Randolph, R. B. F.	- - - -	Trenton, N. J.
Wesbrook, E. F.	- - - -	Minneapolis, Minn.
Winslow, C. E. A.	- - - -	Boston, Mass.

#### LIST OF THOSE WHO TOOK PART IN THE COMPARATIVE STUDIES OF BACTERIAL SPECIES.

Chester, F. D.	- - - -	Newark, Del.
Conn, H. W.	- - - -	Middletown, Conn.
Copeland, William R.	- - - -	Columbus, O.
De Schweinitz, E. A.	- - - -	Washington, D. C.
Gage, S. DeM.	- - - -	Lawrence, Mass.
Gehrmann, Adolph	- - - -	Chicago, Ill.
Gorham, Fred. P.	- - - -	Providence, R. I.
Hill, H. W.	- - - -	Boston, Mass.
Horton, Elmer G.	- - - -	Columbus, O.

Johnson, George A.	- - -	Columbus, O.
Johnston, Wyatt	- - -	Montreal, Que.
Jordan, E. O.	- - -	Chicago, Ill.
Laird, A. T.	- - -	Albany, N. Y.
McFarland, Joseph	- - -	Philadelphia, Pa.
Parker, H. N.	- - -	Washington, D. C.
Russell, H. L.	- - -	Madison, Wis.
Smith, Theobald	- - -	Boston, Mass.
Stokes, W. R.	- - -	Baltimore, Md.
Stone, B. H.	- - -	Burlington, Vt.
Weston, R. S.	- - -	Boston, Mass.
Whipple, George C.	- - -	New York, N. Y.
Wilson, Ezra H.	- - -	Brooklyn, N. Y.

## LIST OF THOSE WHO TOOK PART IN THE COMPARISON OF CULTURE MEDIA.

Amyot, J. A.	- - -	Toronto, Ont.
Burrage, Severance	- - -	Lafayette, Ind.
Chester, F. D.	- - -	Newark, Del.
Copeland, W. R.	- - -	Columbus, O.
Gage, S. DeM.	- - -	Lawrence, Mass.
Gorham, Fred. P.	- - -	Providence, R. I.
Hill, Hibbert W.	- - -	Boston, Mass.
Hiss, Philip, Jr.	- - -	New York, N. Y.
Horton, E. G.	- - -	Columbus, O.
Jordan, E. O.	- - -	Chicago, Ill.
McFarland, J. W.	- - -	Philadelphia, Pa.
Park, W. H.	- - -	New York, N. Y.
Randolph, R. B. F.	- - -	Trenton, N. J.
Russell, H. L.	- - -	Madison, Wis.
Walters, E. P.	- - -	Boston, Mass.
Wesbrook, F. F.	- - -	Minneapolis, Minn.
Whipple, George C.	- - -	New York, N. Y.

## COLLECTION OF SAMPLES.

## QUANTITY OF WATER REQUIRED FOR ANALYSIS.

The minimum quantity necessary for making the ordinary physical, chemical, and microscopical analyses of water or sewage is one gallon; for the bacteriological examination, two ounces. In special cases larger quantities may be required.

## BOTTLES.

The bottles for the collection of samples shall be made of hard, clear, white glass, and shall have glass stoppers. Cork stoppers shall not be permitted except when physical or microscopical examinations only are to be made. Earthen jugs or metal containers shall not be used.

Sample bottles shall be carefully cleansed each time before using. This may be done by treating with sulphuric acid and potassium bichromate, or with alkaline permanganate and afterwards with a mixture of oxalic and sulphuric acids, and by thoroughly rinsing with water and draining.

When clean, the stoppers and necks of the bottles shall be protected from dirt by tying cloth or thick paper over them.

For shipment they shall be packed in cases with a separate compartment for each bottle. Wooden boxes may be lined with indented fibre paper, felt, or some similar substance, or provided with spring corner strips, to prevent breakage. Lined wicker baskets also may be used.

Bottles for bacterial samples, besides being washed, shall be sterilized with dry heat for one hour at  $160^{\circ}\text{C.}$ , or in an autoclave at  $115^{\circ}\text{C.}$  for fifteen minutes. For transportation they may be wrapped in sterilized cloth or paper, or the necks may be covered with tin-foil and the bottles put in tin boxes. When bacterial samples must of necessity stand for twelve hours before plating, bottles holding more than four ounces shall be used.

The bottles used for chemical samples may be sterilized and the samples so collected used for the bacteriological analysis. When bacterial samples are not plated at the time of collection they shall be kept on ice at a temperature of not more than  $15^{\circ}\text{C.}$ , and preferably as low as  $10^{\circ}\text{C.}$

#### TIME INTERVAL BETWEEN COLLECTION AND ANALYSIS.

Generally speaking, the shorter the time elapsing between the collection and the analysis of a sample, the more reliable will be the analytical results. Under many conditions, analyses made in the field are to be commended, as data so obtained are frequently preferable to those made in a distant laboratory after the composition of the water has changed en route.

The allowable time that may elapse between the collection of a sample and the beginning of its analysis cannot be stated definitely, as it depends upon the character of the sample and upon other conditions, but the following may be considered as fairly reasonable maximum limits under ordinary conditions:

#### *Physical and Chemical Analysis.*

Ground waters	72 hours
Fairly pure surface waters	48 "
Polluted surface waters	12 "
Sewage effluents	6 "
Raw sewages	6 "

*Microscopical Examination.*

Ground waters	-	-	-	-	-	-	-	-	72 hours
Fairly pure surface waters	-	-	-	-	-	-	-	-	24 "
Waters containing fragile organisms	-								Immediate examination

*Bacteriological Examination.*

Ground waters	-	-	-	-	-	-	-	-	6 hours
Fairly pure surface waters	-	-	-	-	-	-	-	-	6 "
Polluted surface waters	-	-	-	-	-	-	-	-	6 "
Sewage effluents	-	-	-	-	-	-	-		Immediate plating
Raw sewages	-	-	-	-	-	-	-		" "

If sterilized by the addition of chloroform, formaldehyde, mercuric chloride, or some other disinfectant, samples for chemical and microscopical examination may be allowed to stand for longer periods than those indicated, but as this is a matter which must vary according to local circumstances, no definite procedure is recommended.

If unsterilized samples of sewage, sewage effluents, and highly polluted surface waters are not analyzed on the day of their collection, caution must be used in regard to the organic contents, which frequently change materially upon standing.

The gaseous contents of samples, especially dissolved oxygen, and carbonic acid should be obtained immediately, in accordance with the directions given beyond in connection with each determination.

## REPRESENTATIVE SAMPLES.

Care shall be taken to secure a sample which is truly representative of the liquid to be analyzed. In the case of sewages this is especially important, in view of the marked variations in composition which occur from hour to hour. Frequently satisfactory samples can be obtained only by mixing together several portions collected at different times or at different places—the details as to collection and mixing depending upon local conditions.

## PHYSICAL EXAMINATION.

## TEMPERATURE.

The temperature of the sample shall be taken at the time of collection, and shall be preferably expressed in Centigrade degrees, to the nearest 0.5 degree or closer if for any reason more



exact data are required. For obtaining the temperature of water at various depths below the surface the thermophone<sup>6</sup> is recommended.

#### TURBIDITY.

The turbidity of water is due to suspended matter, such as clay, silt, finely divided organic matter, microscopic organisms, etc. The increasing use of filters for the purification of water and sewage has made this determination one of great importance.

#### TURBIDITY STANDARD.

The standard of turbidity shall be that adopted by the United States Geological Survey, namely, a water which contains 100 parts of silica per million in such a state of fineness that a bright platinum wire one millimeter in diameter can just be seen when the center of the wire is 100 millimeters below the surface of the water and the eye of the observer is 1.2 meters above the wire, the observation being made in the middle of the day, in the open air, but not in sunlight, and in a vessel so large that the sides do not shut out the light so as to influence the results. The turbidity of such water shall be 100.

#### COEFFICIENT OF FINENESS.<sup>7</sup>

The number obtained by dividing the weight of suspended matter in the sample (in parts per million) by the turbidity shall be called the coefficient of fineness. If greater than unity it indicates that the matter in suspension in the water is coarser than the standard; if less than unity, that it is finer than the standard.

#### PREPARATION OF SILICA STANDARD.<sup>8</sup>

Use diatomaceous earth as free as possible from sponge spicules and amorphous silica. Wash with water to remove soluble salts; dry, and ignite to remove organic matter; treat and warm with dilute hydrochloric acid; wash with distilled water until free of acid, and dry thoroughly.

Grind in an agate mortar, sifting through a No. 200 mesh sieve in order to separate mats obtained by grinding, and dry in a desiccator.

One gram of this preparation in one liter of distilled water makes a stock suspension which contains 1000 parts per million of

silica, and which should have a turbidity of 1000. Test this suspension, after diluting a portion of it with nine times its volume of distilled water, with a wire to ascertain if the silica has the necessary degree of fineness, and if the suspension has the necessary degree of turbidity. If not, correct by adding more silica or more water as the case demands.\*

Standards for comparison shall be prepared from this stock suspension by dilution with distilled water. For turbidity readings below 20, standards of 0, 5, 10, 15 and 20 shall be kept in gallon bottles made of clear white glass; for readings above 20, standards of 20, 30, 40, 50, 60, 70, 80, 90 and 100 shall be kept in 100 c.c. nessler tubes, approximately 20 millimeters in diameter.

Comparison of the water under examination with the standards shall be made by viewing them sidewise toward the light, looking at some object and noting the distinctness with which the margins of the object can be seen.

The standards shall be kept stoppered, and both sample and standards shall be thoroughly shaken before making the comparison.

In order to prevent any bacterial or algal growths from appearing in the standards, a small amount of bichloride of mercury may be added to them.

#### PLATINUM WIRE METHOD.<sup>9</sup>

This method requires a rod with a platinum wire of a diameter of one mm. or 0.04 inch inserted in it about one inch from the end of the rod, and projecting from it at least one inch at a right angle. Near the end of the rod, at a distance of 1.2 meters (about four feet) from the platinum wire, a wire ring shall be placed directly above the wire through which, with his eye directly above the ring, the observer shall look when making the examination. The rod shall be graduated as follows:

The graduation mark of 100 shall be placed on the rod at a distance of 100 mm. from the center of the wire. Other graduations shall be made according to Table 2, which is based on the best obtainable data and in which the distances are intended to be such that when the water is diluted the turbidity readings will

\*This method of correction very slightly alters the coefficient of fineness of the standard, but does not noticeably affect its use.



decrease in the same proportion as the percentage of the original water in the mixture. These graduations are those used to construct what is known as the U. S. Geological Survey Turbidity Rod of 1902.<sup>10</sup>

TABLE 2.

Turbidity. Parts per Million	Vanishing Depth of Wire mm.	Turbidity. Parts per Million	Vanishing Depth of Wire mm.
7	1095	70	138
8	971	75	130
9	873	80	122
10	794	85	116
11	729	90	110
12	674	95	105
13	627	100	100
14	587	110	93
15	551	120	86
16	520	130	81
17	493	140	76
18	468	150	72
19	446	160	68.7
20	426	180	62.4
22	391	200	57.4
24	361	250	49.1
26	336	300	43.2
28	314	350	38.8
30	296	400	35.4
35	257	500	30.9
40	228	600	27.7
45	205	800	23.4
50	187	1000	20.9
55	171	1500	17.1
60	158	2000	14.8
65	147	3000	12.1

*Procedure.*—Push the rod down into the water vertically as far as the wire can be seen and then read the level of the surface of the water on the graduated scale. This will indicate the turbidity.

The following precautions shall be taken to insure correct results:

Observations shall be made in the open air, preferably in the middle of the day and not in direct sunlight. The wire shall be kept bright and clean. If for any reason observations cannot be made directly under natural conditions, a pail or tank may be filled with water and the observation taken in that, but in this case care shall be taken that the water is thoroughly stirred before the observation is made, and no vessel shall be used for this purpose unless its

diameter is at least twice as great as the depth to which the wire is immersed. Waters which have a turbidity above 500 shall be diluted with clear water before the observations are made, but in case this is done the degree of dilution used shall be stated and form a part of the report.

The wire method shall be used for testing the degree of fineness of the standard silica, and this degree of fineness shall be such that when added to distilled water in an amount equal to 100 parts per million, the wire observed under standard conditions can be just seen at a depth of 100 mm. below the surface of the water.

#### TURBIDIMETRIC METHOD.

Several forms of turbidimeters (or diaphanometers<sup>11</sup>) have been suggested for use, but as improvements in them are being constantly made no definite form is here prescribed for use. The simplest and most satisfactory form at present is the candle turbidimeter.<sup>12</sup> \*

This consists of a graduated glass tube with a flat polished bottom, enclosed in a metal case. This is held over an English standard candle and so arranged that one may look vertically down through the tube and see the image of the candle. The observation is made by pouring the sample of water into the tube until the image of the candle just disappears from view. Care shall be taken not to allow soot or moisture to accumulate on the lower side of the glass bottom of the tube so as to interfere with the accuracy of the observations. The graduations on the tube correspond to turbidities produced in distilled water by certain numbers of parts per million of silica standard. In order to insure uniform results it is necessary to have the distance between the top rim of the candle and the bottom of the tube constant, and this distance shall be three inches, or 7.6 cm. The observations shall be made in a darkened room or with a black cloth over the head.

All apparatus of this description shall be calibrated to correspond with the United States Geological Survey scale. The following figures are believed to be approximately correct for the candle turbidimeter, but should be checked by the experimenter.

\* Manufactured by Baker and Fox, 83 Schermerhorn St., Brooklyn, N. Y.

TABLE 3.

Depth in Centimeters	Turbidity Parts per Million of Silica	Depth in Centimeters	Turbidity Parts per Million of Silica
2.3	1000	7.5	290
2.6	900	7.8	280
2.9	800	8.1	270
3.2	700	8.4	260
3.5	650	8.7	250
3.8	600	9.1	240
4.1	550	9.5	230
4.5	500	9.9	220
4.9	450	10.3	210
5.5	400	10.9	200
5.6	390	11.4	190
5.8	380	12.0	180
5.9	370	12.7	170
6.1	360	13.5	160
6.3	350	14.4	150
6.4	340	15.4	140
6.6	330	16.6	130
6.8	320	18.0	130
7.0	310	19.6	110
7.3	300	21.5	100

## EXPRESSION OF RESULTS.

The results of turbidity observations shall be expressed in whole numbers which correspond to parts per million of silica, and recorded as follows:

Turbidity between	1 and	50	Recorded to nearest unit
" "	51	100	" " " 5
" "	101	500	" " " 10
" "	501	1000	" " " 50
" "	1001	above	" " " 100

## COLOR.

The "color" of water, or the "true color," shall be considered as that part of the apparent color which is due only to substances in solution; that is, it is the color of the water after the suspended matter has been removed.

The "apparent color" shall be considered as including not only the true color but also any color produced by substances in suspension. It is the color as viewed by inspection of the original sample.

In stating the results the word "color" shall mean the "true color" unless otherwise expressed.

The platinum-cobalt method of measuring color shall be con-

sidered as the standard, and the unit of color shall be that produced by one part per million of platinum.

PLATINUM-COBALT STANDARD.<sup>13</sup>

The standard solution, which has a color of 500, shall be prepared as follows :

Dissolve 1.246 grams of potassium platinic chloride ( $\text{PtCl}_4 \cdot 2\text{KCl}$ ) containing 0.5 gram platinum, and one gram crystallized cobalt chloride ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ) containing 0.25 gram of cobalt in water, with 100 c.c. concentrated hydrochloric acid, and make up to one liter with distilled water.

By diluting this solution with distilled water to the 100 c.c. graduation mark on the nessler tubes, standards shall be prepared having colors of 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60 and 70. These shall be kept in nessler tubes of such diameter that the 100 c.c. graduation mark is between 20 and 25 cm. above the bottom and is uniform for all tubes. They shall be protected from dust when not in use.

*Procedure.*—The color of a sample shall be observed by filling a standard nessler tube to the graduation mark with the water to be examined to a depth equal to that of the standards and by comparing it with the standards. The observation shall be made by looking vertically downwards through the tubes upon a white surface placed at such an angle that light is reflected upwards through the column of liquid.

Waters that have a color darker than 70 shall be diluted before making the comparison, in order that no difficulties may be encountered in matching the hues.

Water containing matter in suspension shall be filtered, before the color observation is made, until no visible turbidity remains. If the suspended matter is coarse, filter paper may be used for this purpose; if the suspended matter is fine, the use of a Berkefeld filter is recommended. The Pasteur filter shall not be used, as it exerts a marked decolorizing action.

The apparent color shall be determined on the original sample without filtration. In the case of clear waters or waters with low turbidities, the true color and the apparent color are substantially the same.

## EXPRESSION OF RESULTS.

The results of color determination shall be expressed in whole numbers and not in hundredths, as was formerly<sup>1</sup> the case, and recorded as follow:

Color between	1 and	50	Recorded to nearest unit
"	"	51 "	100
"	"	101 "	250
"	"	251 "	500
			5
			10
			20

U. S. GEOLOGICAL SURVEY FIELD METHOD.<sup>10</sup>

As the above described method is not well adapted for field work, a method has been devised by which the color of the water to be tested may be compared with that of glass disks\* held at the end of metallic tubes through which they are viewed by looking towards a white surface. When this method is used the glass disks are individually calibrated (by the makers\*) to correspond with colors on the platinum scale. Experience has shown that the glass disk method used by the U. S. Geological Survey gives results in substantial agreement with those obtained by the platinum determinations, and its use is recognized as a standard procedure.

## COMPARISON WITH NESSLER STANDARDS.

Inasmuch as the nessler scale<sup>14</sup> (and the natural water scale,<sup>15</sup> which agrees with it except for the figures below 20) has been largely used in the past, it is often desirable to transpose<sup>16</sup> the old results to the platinum standard. For this purpose the ratios given in Table 4 may be conveniently employed, but they must not be considered as universally applicable, as the character of the nessler solution introduces an uncertain factor.

## RECORDS OF TINTS AND SHADES OF APPARENT COLOR.

The value of the readings of tint and shade by the Lovibond Tintometer<sup>17</sup> has not been commensurate with the labor involved, but it is necessary in some cases to make a record of the reflected tint and shade<sup>18</sup> of the water.

The standard color disks† used in teaching optics may be used for the purpose.

\*This apparatus is made by the Builders Iron Foundry, Providence, R. I.

†Manufactured by the Milton Bradley Educational Co., Springfield, Mass.



TABLE 4.

TABLE FOR CONVERTING COLORS BY THE NATURAL WATER SCALE INTO PARTS PER MILLION OF COLOR BY THE PLATINUM STANDARD.\*

Modified Nessler or Natural-Water Standard	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.00	0	2	4	6	8	9	11	13	15	17
0.10	18	19	20	21	22	23	24	24	26	26
0.20	26	27	27	28	29	29	30	31	32	32
0.30	33	34	34	35	35	36	37	37	38	38
0.40	39	40	40	41	42	42	43	44	45	45
0.50	46	47	47	48	48	49	50	50	51	51
0.60	52	53	53	54	54	55	56	56	57	57
0.70	58	58	59	59	60	60	61	61	62	62
0.80	63	64	64	65	66	66	67	68	69	69
0.90	70	71	72	73	74	75	77	78	79	80
1.00	81	82	82	83	84	84	85	86	87	87
1.10	88	89	89	90	91	91	92	93	94	94
1.20	95	96	96	97	98	98	99	100	101	101
1.30	102	103	103	104	105	105	106	107	108	108
1.40	109	110	110	111	112	112	113	114	115	115
1.50	116	117	117	118	118	119	120	120	121	121
1.60	122	123	123	124	125	125	126	127	128	128
1.70	129	130	130	131	132	132	133	134	135	136
1.80	136	137	137	138	139	139	140	141	142	142
1.90	143	144	144	145	146	146	147	148	149	149
2.00	150	...	...	...	...	...	...	...	...	...

*Procedure.*—The white disk supports three movable standard color sectors, red, yellow and blue, also one movable black sector. The whole is mounted on a device which can be revolved rapidly and which blends the colors into a uniform tint or shade. Around the circumference of the disk there is a scale which is used to indicate the percentage of each color or white or black in the blend.

Place the sample in a battery jar on a white background; adjust the sectors so that when blended the tint or shade will match the reflected tint or shade of the sample. Express the results as the percentages of red, yellow, blue, white and black in the blended tint or shade.

#### ODOR.<sup>19</sup>

The observation of the odor of cold and hot samples of surface waters is very important, as the odors are usually connected with some organic growths or with sewage contamination, or both.

The odor of ground waters is often caused by the earthy constituents of the water-bearing strata. The odor of a contaminated well water is often decisive evidence of its pollution.

\* The zero on the true Nessler scale reads about 15 on the platinum scale.



A study of the organisms as directed under Microscopical Examination, page 80, is an invaluable adjunct to the physical and chemical examination of water. Certain organisms can be distinguished by their odor, as, for example, the "fishy" odor of *Uroglena*, the "aromatic" or "rose geranium" odor of *Asterionella* and the "pig-pen" odor of *Anabaena*.

*Procedure*.—Observe and record the odor, both at room temperature and at just below the boiling-point, as follows:

*Cold Odor*.—Shake the sample violently in one of the gallon collecting bottles, when it is from about half or two-thirds full and when the sample is at room temperature (about 20° C.). Remove the stopper and smell the odor at the mouth of the bottle.

*Hot Odor*.—Into a tall 400 c.c. beaker without lip pour about 150 c.c. of the sample. Cover the beaker with a well-fitting watch glass, place on a hot plate and bring the water to just below boiling. Remove the beaker from the plate and allow it to cool for not more than five minutes. Then shake with a rotary movement, slip the watch glass to one side and smell the odor.

#### EXPRESSION OF RESULTS.

Express the quality of the odor by some such descriptive epithet as the following, which for purposes of record may be abbreviated:

v—vegetable.	m—moldy.
a—aromatic.	M—musty.
g—grassy.	d—disagreeable.
f—fishy.	p—peaty.
e—earthy.	s—sweetish.

Express the intensity of the odor by a numeral prefixed to the term expressing quality, which may be defined as follows:

Numerical Value.	Term.	Approximate Definition.
0	None.	No odor perceptible.
1	Very faint.	An odor that would not be ordinarily detected by the average consumer, but that could be detected in the laboratory by an experienced observer.
2	Faint.	An odor that the consumer might detect if his attention were called to it, but that would not otherwise attract attention.

Numerical Value.	Term.	Approximate Definition.
3	Distinct.	An odor that would be readily detected and that might cause the water to be regarded with disfavor.
4	Decided.	An odor that would force itself upon the attention and that might make the water unpalatable.
5	Very strong.	An odor of such intensity that the water would be absolutely unfit to drink. (A term to be used only in extreme cases.)

## CHEMICAL EXAMINATION.

The scope of the chemical determinations considered in this report is sufficiently indicated by the table of contents, and the general applicability of each determination according to the views of the committee is set forth in the letter of transmittal. No further introduction is required except to refer to the brief description of the nature and purpose of each determination which precedes the various methods given.

## EXPRESSION OF RESULTS.

The committee recommends that the results of chemical analyses be expressed in parts per million, which in most cases is practically equivalent to milligrams per liter. Nevertheless it is recognized that in some of the older laboratories, where for many years other forms of expression have been used, it may not be expedient to change their present practice at once.

The results expressed in parts per 100,000 or in grains per gallon may be transformed to parts per million, or conversely, by the use of the following table:

TABLE 5.

	Grains per U. S. Gallon	Grains per Imperial Gallon	Parts per 100,000	Parts per 1,000,000
1 grain per U. S. gallon .....	1.000	1.20	1.71	17.1
1 grain per Imperial gallon .....	0.835	1.00	1.43	14.3
1 part per 100,000 .....	0.585	0.70	1.00	10.0
1 part per 1,000,000 .....	0.053	0.07	0.10	1.0

The committee desires to call attention to the practice of many analysts of using too many decimals in expressing the results of

analyses. In some instances the records imply that the methods of sampling and analysis are 10 or even 100 times more accurate than the facts warrant. The following general rules are advised:

1. When the results show quantities above 10 parts per million, do not use any decimals; record only whole numbers. In fact, where the quantities reach hundreds and thousands of parts, there is much merit in recording a cipher in the units place, and in following the general manner of expression given under Turbidity, page 20.

2. When the results are between 1 and 10 parts, do not carry decimals to more than one place.

3. When the results are between 0.1 and 1 part, do not carry decimals to more than two places.

4. Nitrogen in drinking waters as free and albuminoid ammonias, and as nitrite, alone justify the use of three decimals.

Where there are tabulated together the results of various analyses calling for two or more of the above rules to be applied in the same column, the committee suggests that if desired ciphers and not digits be used at the right of the larger figures to make the column uniform in appearance.

#### DETERMINATION OF OXYGEN CONSUMED.<sup>20</sup>

“Oxygen consumed” means the oxygen which the organic compounds of sewage and waters consume when treated in an acid solution with potassium permanganate. The expression is synonymous with “oxygen required” and with “oxygen absorbed.” The expression “dissolved oxygen” refers to another determination.

It is the carbon, and not the nitrogen, in organic matter which is oxidized in this way by potassium permanganate, hence this determination is frequently referred to as an indication of the carbonaceous organic matter present. However, it indicates only a certain portion of the carbon, and this ratio varies in different samples of water and of sewage. Furthermore, it does not differentiate the carbon present in unstable organic matter from that in what might be called fairly stable organic matter, such as is sometimes referred to as residual humus matter.

If nitrites, ferrous iron, sulphides or other unoxidized mineral compounds are present they will increase the oxygen consumed; and hence a correction should be made for them when studying carbonaceous organic matter.

It is one of the oldest methods for determining organic matter, and in fact has been in very wide use for more than half a century. Its introduction followed the recognition of the fact that the loss on ignition of the residue upon evaporation may indicate certain volatile mineral matters as well as organic matter. Unfortunately this determination has been made by a great variety of procedures as to certain details, and these variations have been detrimental to establishing the method on the most favorable basis.

The essential features of the method common to all the various procedures will be first described, after which will be taken up the variable factors, namely, the temperature and time that the permanganate solution is allowed to act on the sample under examination.

*Reagents.*—1. Dilute sulphuric acid. One part of sulphuric acid to three parts of distilled water. This shall be freed from oxidizable matters by adding potassium permanganate until a faint pink color persists after standing several hours.

2. Standard potassium permanganate solution. Dissolve 0.4 gram of the crystalline compound in one liter of distilled water. Standardize against an ammonium oxalate solution. One c.c. is equivalent to 0.0001 gram of available oxygen.

3. Ammonium oxalate solution. Dissolve 0.888 gram of the substance in one liter of distilled water. One c.c. is equivalent to 0.0001 gram of oxygen.

4. Potassium iodide solution. Ten per cent solution free of iodate.

5. Sodium thiosulphate solution. Dissolve 1.0 gram of the pure crystallized salt in one liter of distilled water. Standardize against a potassium permanganate solution which has been standardized against an ammonium oxalate solution. As this solution does not keep well, determine its actual strength at frequent intervals.



6. Starch indicator. Prepare as directed beyond under Dissolved Oxygen, page 74.

*Procedure.*—Measure into a flask 100 c.c. of the water, or a smaller diluted portion if the water is of high organic content. Add 10 c.c. of sulphuric acid solution and 10 c.c. of potassium permanganate solution, and allow the treated sample of water to digest in accordance with the detailed procedures carried out under differing conditions as set forth below under the heading Period and Temperature of Digestion, page 29.

Precisely at the end of the period of digestion, remove the flask, and if the boiling temperature is used, add 10 c.c. of the ammonium oxalate solution. Titrate with the permanganate solution until a faint but distinct color is obtained.

Each c.c. of the permanganate solution in excess of the oxalate solution represents 0.0001 gram of oxygen consumed by the sample.

At the end of the period of digestion, if not made at the boiling temperature, add 0.5 c.c. of potassium iodide solution to discharge the pink color; mix; titrate the liberated iodine with thiosulphate until the yellow color is nearly destroyed, then add a few drops of starch solution and continue titration until the blue color is just discharged.

Should the volume of permanganate solution be insufficient for complete oxidation, repeat the analysis, using a larger volume so that at least three c.c. of the permanganate solution will be present in excess when the ammonium oxalate solution is added.

When unoxidized mineral substances, such as ferrous sulphate, sulphides, nitrites, etc., are present in the sample, corrections should be applied as accurately as possible by procedures suitable for the samples being analyzed. Direct titration of the acidified sample in the cold, using a three minute period of digestion, serves this purpose quite well for polluted surface waters and fairly well for purified sewage effluents. Raw sewages containing no trade wastes seldom need such a correction; but when raw sewages contain "pickling liquors" it is important. In all samples containing both unoxidized mineral compounds and gaseous organic substances, the latter should be driven off by heat and the sample

allowed to cool before applying this test for the correction factor. Where such corrections are necessary the fact should be stated, with the amount of correction.

*Period and Temperature of Digestion.*—Unfortunately, widely varying details are practiced in this regard. This means that it is difficult to compare the results obtained at one laboratory with those obtained at another; and, furthermore, the amount of data obtained at various places is so great that it is awkward and difficult to make a change at some laboratories, as future data obtained by modified methods will not be directly comparable with existing data obtained by the present methods. None of the methods gives absolute results; they are only relative<sup>21</sup> at best. The principal methods may be mentioned as follows:

1. Bring the acidified sample to the boiling point, add the permanganate solution and digest for two minutes<sup>22</sup> at a boiling temperature. This procedure is facilitated by agitating the liquid constantly with a small current of air to guard against bumping.

2. Same method as No. 1 except that the period of digestion is five minutes<sup>23</sup> instead of two minutes.

3. Same method as No. 2 except that the permanganate solution is added to the acidulated sample when cold, and the period of digestion continued for five minutes after the sample reaches the boiling point. The advantage of this method is that there are recorded the oxygen consuming powers of the volatile matters present in some sewages and sewage effluents, which are driven off by heat and thus escape when analyses are made in accordance with the first two procedures above noted.

4. Same method as No. 3 except that the period of digestion is 10 minutes<sup>24</sup> instead of five minutes.

5. The permanganate is added in the cold to the acidulated sample and the flask placed immediately in a bath of boiling water, the water level of which is kept above the level of the contents in the flask. Digestion is continued for exactly 30 minutes.<sup>25</sup>

6. Digestion of the sample after the acid and permanganate solutions are added is carried out abroad, especially in England, at approximately the room temperature,<sup>26</sup> apparently to guard against decomposition<sup>27</sup> of permanganate in the presence of high



chlorine, for periods of three minutes, 15 minutes and four hours; many observers record the oxygen consumed after all three periods, while some record the result only for the four-hour period.

*Concluding Notes.*—After careful consideration of the matter the views of the committee are as follows:

1. From a strictly scientific standpoint the 30-minute period of digestion at boiling temperature in a water bath appears to give the most satisfactory results as regards uniformity and freedom from personal errors of manipulation. It is believed that where practicable this is the most satisfactory method for adoption.

2. Where samples from a given source are repeatedly analyzed it is advised that there be placed on record, for purposes of comparison, representative results by each of the methods of digestion above mentioned.

3. In connection with sewage works analysis, to which the usefulness of this method is principally confined (see Table 1), it is recommended that the permanganate solution be added to the sample before heating in order to include the oxygen consumed by volatile compounds.

#### DETERMINATION OF NITROGEN.

Nitrogenous organic matter, due to natural agencies, passes from crude organic matter through several intermediate compounds and (that which does not gasify) ultimately forms nitrates.

Nitrogen in the form of organic matter can be determined as organic nitrogen by the so-called Kjeldahl process,<sup>28</sup> and it can be also approximated by the albuminoid ammonia determination.<sup>29</sup> It is not possible to differentiate the nitrogen in the organic matter which readily decomposes from that in stable or non-putrescible compounds.

Decomposition of organic matter produces nitrogen in the form of free or saline ammonia, which is the first intermediate step between crude nitrogenous organic matter and the completely mineralized matter in the form of nitrates. Nitrogen as free ammonia may be determined by distillation and nesslerization, or by the direct nesslerization of the clarified sample.

The second intermediate form in which nitrogen occurs is that of nitrites.

When nitrogenous organic matter is completely mineralized, as above stated, it is present in the form of nitrates.

The importance of the determination of nitrogen in working with sewage and unpurified water supplies causes this set of determinations to be of much significance. They are described at length, from the standpoint of regular laboratory practice, supplemented in some particulars from the standpoint of field work such as is required in the operation of sewage works.

#### NITROGEN AS FREE AMMONIA.

There are two methods for estimating nitrogen as free ammonia, namely, (A) by distillation, and (B) by direct nesslerization.

The former is recommended for general use, although for sewages, sewage effluents and highly polluted surface waters the great convenience and apparent adequacy of the latter are fully recognized. It is further believed that the slight loss in ammonia by direct nesslerization, perhaps 10 per cent on an average, is probably no more serious and perhaps less so than the inclusion by distillation of intermediate compounds, which do not strictly belong to nitrogen as free ammonia. The difficulty, however, of obtaining a proper treatment of some samples so that they will not become cloudy when nesslerized, makes this procedure, in the opinion of some, rather too uncertain for general adoption at this time. Nevertheless, it is the opinion of the committee that more thorough study will cause the apparent objection to direct nesslerization to disappear, and that wider familiarity with this method will soon bring it into general use for sewage work, as is now the case to some degree abroad<sup>30</sup> and at a few places in this country.

For waters low in free ammonia it is now somewhat uncertain what will be the future of the direct nesslerization method.

#### *Free Ammonia by Distillation.*<sup>31</sup>

*Procedure.*—A metal or glass flask, connected to the condenser<sup>32</sup> in such a way that the distillate may be conveniently delivered from the tin or aluminum condenser tubes directly into the nessler tubes, shall be freed from ammonia by boiling distilled water in it, until the distillate shows no further traces of free ammonia. When this has been done, empty the distilling flask

and measure into it 500 c.c. of the sample, or a smaller portion diluted to 500 c.c. Apply heat so that the distillation will be at the rate of not more than 10 c.c. nor less than 6 c.c. per minute.

Collect three nessler tubes of the distillate, 50 c.c. to each portion; these contain the free ammonia to be measured as described below.

If the sample is acid, or if the presence of urea is suspected, add about 0.5 gram of sodium carbonate previous to distillation. Omit this when possible, as it tends to increase "bumping."

Use only nessler tubes which do not show a variation of more than six mm. (0.25 inch) in the distance which the graduation mark (50 c.c.) is above the bottom. The tubes shall be of clear white glass, with polished bottoms.

#### *Measurement of Nitrogen as Ammonia.*

This measurement may be made either by (1) comparison of the nesslerized distillates with nesslerized solutions containing known quantities of nitrogen as ammonium chloride, or by (2) comparison of the nesslerized distillates with permanent standard solutions in which the colors of nesslerized standard ammonia solutions are duplicated by solutions of platinum and cobalt chlorides.

#### *Comparison with Ammonia Standards.*

*Reagents.*—1. Ammonia-free water.

2. Standard ammonium chloride solution. Dissolve 3.82 grams of ammonium chloride in one liter of distilled water; dilute 10 c.c. of this to one liter with ammonia-free water. One c.c. equals 0.00001 gram of nitrogen.

3. Nessler's reagent. Dissolve 50 grams potassium iodide in a minimum quantity of cold water. Add a saturated solution of mercuric chloride until a slight but permanent precipitate persists. Add 400 c.c. of 50 per cent solution of potassium hydrate, made by dissolving the potassium hydrate and allowing it to clarify by sedimentation before using. Dilute to one liter, allow to settle and decant.<sup>33</sup> This solution should give the required color with ammonia within five minutes after addition, and should not precipitate with small amounts of ammonia within two hours.

Prepare a series of 16 Nessler tubes which contain the following numbers of c.c. of the standard ammonium chloride solution, diluted to 50 c.c. with ammonia-free water, namely: 0.0, 0.1, 0.3, 0.5, 0.7, 1.0, 1.4, 1.7, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 6.0. These will contain 0.00001 gram of nitrogen for each c.c. of the standard solution used.

Nesslerize the standards and also the distillates by adding approximately two c.c. of nessler reagent to each tube. Do not stir the contents of the tubes.

Have the temperature<sup>34</sup> of the tubes practically the same as that of the standards, otherwise the colors will not be directly comparable.

Compare the color produced in these tubes with that in the standards by looking vertically downward through them at a white surface placed at an angle in front of a window so as to reflect the light upwards. Allow the tubes to stand for at least 10 minutes after nesslerizing before making the comparison.

In case the color obtained by nesslerizing the distillates is greater than that of the darkest tube of the standards, mix the contents of the tube thoroughly and pour out half of the liquid, making up the remainder to the original volume with ammonia-free water, then make the color comparison and multiply the result by two. If, after pouring out half of the liquid, the color is still too dark, repeat this process of division until a reading can be made.

In case the color of the distillates is too high, this process may be shortened by mixing together all of the distillates from one sample before making the comparison, subsequently taking an aliquot portion for comparing with the standards.

After the readings have been made and recorded, add together the results obtained by nesslerizing each portion of the entire distillate from each sample. If 500 c.c. of the sample are distilled, this sum multiplied by .02 will give the number of parts per million of nitrogen as free ammonia in the sample.

#### *Comparison with Permanent Standards.*<sup>35</sup>

In this method, which is rapidly coming into general use in the large laboratories, the distillates are nesslerized in the same



manner as above described; and the resulting colors at the end of about 10 minutes are compared with permanent standards made as follows:

*Platinum Solution.*—Weigh out two grams of potassium platonic chloride ( $\text{Pt Cl}_4 2 \text{KCl}$ ), dissolve in a small amount of distilled water, add 100 c.c. of strong hydrochloric acid and make up to one liter.

*Cobalt Solution.*—Weigh out 12 grams of cobaltous chloride ( $\text{CoCl}_2 6 \text{H}_2\text{O}$ ), dissolve in distilled water; add 100 c.c. of strong hydrochloric acid and make up to one liter.

Prepare standards by putting varying amounts of these two solutions in nessler tubes filling up to the 50 c.c. mark with distilled water as follows:

TABLE 6.

Equivalent Volume of Standard Ammonium Chloride c.c.	Platinum Solution c.c.	Cobalt Solution c.c.
0.0	1.2	0.0
0.1	1.8	0.0
0.3	2.8	0.0
0.5	4.7	0.1
0.7	5.9	0.2
1.0	7.7	0.5
1.4	9.9	1.1
1.7	11.4	1.7
2.0	12.7	2.2
2.5	15.0	3.3
3.0	17.3	4.5
3.5	19.0	5.7
4.0	19.7	7.1
4.5	19.9	8.7
5.0	20.0	10.4
6.0	20.0	15.0
7.0	20.0	22.0

It is necessary to use tubes which have the 50 c.c. mark not less than 20 nor more than 22 cm. above the bottom.

These standards may be kept for several months if protected from dust.

The method of calculating results is precisely the same as with the ammonia standards.

#### *Modification of the Distillation Process for Sewages.*

The determination of free and albuminoid ammonia in sewages, soils and other material of high nitrogen content may be satis-



factorily made by first diluting the sample with ammonia-free distilled water, and proceeding as above described; but it is generally preferable, especially in connection with albuminoid ammonia tests, to use the steam method,<sup>36</sup> as follows:

*Procedure.*—Put the sample to be tested, after the entire apparatus is freed from ammonia, in a long Kjeldahl flask which has a capacity of about 200 c.c., using such an amount of the sample that the color of the nesslerized distillates will fall within the range of the standards.

Generate ammonia-free steam in an ordinary distilling flask and pass it through the liquid in the Kjeldahl flask by means of a glass tube which extends almost to the bottom of the latter. Connect the neck of the Kjeldahl flask with the condenser in the usual way.

This method has the advantage of yielding the free ammonia and also the albuminoid ammonia, when the sample is treated with an alkaline solution of permanganate, more promptly than by the ordinary process of distillation; and, furthermore, “bumping” is avoided. It also permits the assay of solid matter.

It is often convenient to collect the free ammonia distillate in a single receptacle and to take an aliquot part of it for nesslerization.

*Free Ammonia by Direct Nesslerization.*<sup>37</sup>

*Reagents.*—1. A 10 per cent solution of copper sulphate.

2. A 10 per cent solution of lead acetate.

3. A 50 per cent solution of sodium or potassium hydrate.

4. A 10 per cent solution of magnesium chloride.

*Procedure (1) for Sewage.*<sup>38</sup>—Fifty c.c. of the sample to be tested are mixed with an equal volume of water, placed in a short nessler tube and a few drops of copper sulphate solution added. After a thorough mixing, one c.c. of the potassium hydrate solution is added and the contents are again thoroughly mixed. The tube is then allowed to stand for a few moments, when a heavy precipitate should fall to the bottom, leaving a colorless supernatant liquid. Nesslerize an aliquot portion of this clear liquid.

*Procedure (2) for Sewage.*<sup>39</sup>—In place of adding copper sulphate to sewages of high magnesium content, it has been found that satisfactory clarification and also softening of the sample may be obtained by heating it to 40° C. after mixing with the caustic alkali. The heat causes the bicarbonate of lime to be precipitated and the magnesium to separate as a gelatinous precipitate (hydrate). During cooling, the bottle containing 100 c.c. of the sample should be shaken several times to facilitate the subsidence of the precipitate. Where samples are low in magnesium content this treatment may be accomplished by adding a small quantity of magnesium chloride.

Many samples containing hydrogen sulphide require the use of lead acetate in addition to the copper, and others require a few trials before the right combination of the three solutions to bring about the best results can be made. In view of the fact that flocculent precipitates absorb varying amounts of ammonia from solution under certain conditions, it is recommended that the smallest practicable amounts of precipitants be used.

The amount of nitrogen as free ammonia is computed after comparisons with standards in the same manner as in the distillation procedure.

#### NITROGEN AS ALBUMINOID AMMONIA.

The addition of an alkaline permanganate solution to liquids containing nitrogenous organic matter causes the formation of ammonia, the amount of which can be measured upon distillation of the treated sample and the nesslerization of the distillate. In sewages and other liquids and substances containing considerable nitrogenous organic matter the percentage of ammonia-forming organic matter is decidedly variable.<sup>40</sup> For this reason albuminoid ammonia results in such cases are less valuable<sup>41</sup> than the total organic nitrogen, called by some the Kjeldahl nitrogen. Hence for sewage work, including the analyses of both the influents and effluents of purification plants, as well as the study of highly polluted streams, it is recommended that albuminoid ammonia determinations be omitted and in their place the total organic nitrogen be determined.

For ground waters and surface waters containing but little

pollution, the nitrogen as albuminoid ammonia quite uniformly approximates about one-half of the total organic nitrogen. Accordingly the continuance of albuminoid ammonia determinations for this class of work is approved. Nevertheless the inferiority of such results to those of total organic nitrogen is recognized.

The method shall be applied in conjunction with that for free ammonia, as follows:

*Reagents.*—Alkaline potassium permanganate. Pour 1200 c.c. of distilled water into a porcelain dish holding 2500 c.c., boil 10 minutes and turn off the gas. Add 16 grams of C. P. potassium permanganate and stir until dissolved. Then add 800 c.c. of 50 per cent clarified solution of potassium or sodium hydrate and enough distilled water to fill the dish. Boil down to 2000 c.c. Test each batch of this solution for albuminoid ammonia by making a blank determination. Correction should be made accordingly.

*Procedure.*—Interrupt the distillation (made as already described) after the collection of the distillate for free ammonia.

Add 40 c.c. or more of alkaline potassium permanganate and conduct this distillation until at least four portions of 50 c.c. each and preferably five portions of the distillate have been collected in separate tubes.

Have enough permanganate solution present to insure the maximum oxidation of the organic matter. These distillates contain the nitrogen as albuminoid ammonia, measurement of which shall be made as described in connection with nitrogen as free ammonia.

Dissolved nitrogen as albuminoid ammonia may be determined from a sample from which suspended matters are removed by filtration either through filter paper, or, if finely divided matters are present, through a Berkefeld filter.

Suspended nitrogen as albuminoid ammonia may be obtained by taking the difference between the total and dissolved results.

The results shall be expressed as in the case of free ammonia.

#### TOTAL ORGANIC NITROGEN.<sup>42</sup>

The total organic nitrogen, the significance of which has been already described, shall be determined as follows:

Boil 500 c.c. of the sample in a round bottom flask until free of ammonia. This usually requires the loss of about 200 c.c. of the sample, which, if desired, may be collected for the determination of free ammonia.

Add five c.c. of C. P. concentrated sulphuric acid which is free of nitrogen, together with a small piece of ignited pumice. Mix by shaking and place over a flame under a hood.

Digest until copious fumes of sulphuric acid are given off and until the liquid chars and finally becomes colorless. Remove from the flame, add potassium permanganate in small portions until a heavy green precipitate persists in the liquid. Cool. Dilute with about 100 c.c. of ammonia-free water. Neutralize with ammonia-free sodium carbonate solution (10 per cent). Distill off the ammonia, collect in nessler tubes and nesslerize and compare with standards as already described.

In the case of sewage it is preferable to distill the free ammonia, by passing live steam through 100 c.c. or less of the sample. Add five c.c. of sulphuric acid and digest, dilute the colorless digested liquid to 500 c.c. Place 10 c.c. or more of this liquid in a 200 c.c. Kjeldahl distilling flask. Dilute with 100 c.c. of water. Neutralize with 10 c.c. of sodium carbonate solution (10 per cent solution), distill with steam and nesslerize.

In this determination care must be taken to digest thoroughly, to add potassium permanganate to the point of precipitation, to sample carefully after dilution and to add enough sodium carbonate to insure the separation of the ammonia from the precipitated manganese hydrate. Potassium permanganate shall not be added during digestion because it causes loss of nitrogen.

#### NITROGEN AS NITRITES.<sup>43</sup>

The following shall be considered the standard mode of procedure for water and sewages in determining the nitrogen as nitrites, the second intermediate step by which nitrogenous matter passes from crude organic matter to mineral matter (nitrates). Nitrites may also be formed by the reduction of nitrates.

*Reagents.*—1. Sulphanilic acid solution. Dissolve eight grams of the purest sulphanilic acid in 1000 c.c. of 5 N. acetic acid (Sp. Gr. 1.041). This is a practically saturated solution.



2. *a*-amidonaphthalene acetate solution. Dissolve 5.0 grams solid *a*-naphthylamine in 1000 c.c. of 5 N. acetic acid; filter the solution through washed absorbent cotton.

3. Sodium nitrite, stock solution. Dissolve 1.1 gram silver nitrite in nitrite-free water; precipitate the silver with sodium chloride solution and dilute the whole to one liter.

4. Standard sodium nitrite solution. Dilute 100 c.c. of solution (3) to one liter; then dilute 10 c.c. of this solution to one liter with sterilized nitrite-free water; add one c.c. of chloroform and preserve in a sterilized bottle. One c.c. = 0.0000001 gram nitrogen.

*Procedure.*—Measure out 100 c.c. of the decolorized sample (decolorized by adding aluminum hydrate free of nitrite—see under Chlorine), or a smaller portion diluted to 100 c.c., into a nessler tube. These nessler tubes shall be of clear white glass, with the 100 c.c. graduation mark not varying more than six mm. in its distance above the bottom. At the same time make a set of standards by diluting various volumes of the standard nitrite solution in Nessler tubes to 100 c.c. with nitrite-free water, for example, 0, 1, 3, 5, 7, 10, 14, 17, 20 and 25 c.c. Add two c.c. of reagents Nos. 1 and 2 (above) to each 100 c.c. of the sample and to each standard. Mix, allow to stand 10 minutes. Compare the samples with the standards. Do not allow the samples to stand over one-half hour before being compared. Make a blank determination in all cases to correct for the presence of nitrite in the air, the water and the reagents. Dilute all samples which develop more color than the 30 c.c. standard before comparing. Mixing is important.

The solution must be acid. Hydrochloric acid, which until recently was in quite general use in this country for a solvent for the naphthalene, permits satisfactory results to be obtained, but the speed of the reaction is much slower than in the case of acetic acid.<sup>44</sup> For this reason the latter acid is preferred.

When 100 c.c. of the sample are used, then 0.001 times the number of c.c. of the standard gives the parts per million of nitrogen as nitrite.



NITROGEN AS NITRATES.<sup>45</sup>

No single method appears to be applicable to the determination of nitrogen as nitrates in all classes of water, sewages and sewage effluents, and there is no method which is not subject to considerable error.

Where the amount of chlorine in the sample is less than about 30 parts per million, the phenolsulphonic acid method is recommended. When the chlorine is greater than this, as in sewage work, the reduction method is recommended.

The standard mode of procedure for these methods shall be as follows:

*Phenolsulphonic Acid Method for Nitrates.*<sup>46</sup>

*Reagents.*—1. Phenolsulphonic acid. Mix 30 grams of synthetic phenol with 370 grams of C. P. concentrated sulphuric acid in a round-bottom flask. Put this flask in a water bath and support it in such a way that it shall be completely immersed in the water. Heat for six hours.

2. Ammonium hydrate solution diluted with distilled water 1 to 1. Potassium hydrate may be used.

3. Standard nitrate solution. Dissolve 0.72 gram of pure recrystallized potassium nitrate in one liter of distilled water. Evaporate cautiously 10 c.c. of this strong solution on the water bath. Moisten quickly and thoroughly with two c.c. of phenolsulphonic acid and dilute to one liter for the standard solution, one c.c. of which equals .000001 gram of nitrogen.

*Procedure*—Evaporate 20 c.c. or less of the sample in a small porcelain evaporating dish on the water bath, removing it from the bath just before it has come to dryness. Let the last few drops evaporate at room temperature in a place protected from the dust. When the sample is suspected to contain a large amount of nitrate, evaporate less than 20 c.c. If it is suspected to contain but little, evaporate more.

If the sample has a high color, decolorize before evaporating by the use of washed aluminum hydrate, as directed in connection with the chlorine determination.

Add one c.c. of phenolsulphonic acid and rub this quickly and thoroughly over the residue with a glass rod. Add about

10 c.c. of distilled water and stir with a glass rod until mixed. Add enough ammonium hydrate solution (or potassium hydrate if the operation must of necessity be carried on in a room where ammonia distillations are made) to render the liquid alkaline. Transfer the liquid to a 100 c.c. nessler tube and fill the tube to the 100 c.c. mark with distilled water.

If nitrates are present there will be formed a yellow color; this may be compared with permanent standards<sup>35</sup> made for the purpose, and kept satisfactorily for several weeks. The series of standards for comparison shall be made by putting the following quantities of the standard solution into 100 c.c. tubes and making up to the 100 c.c. mark with distilled water, adding five c.c. of strong ammonia to each tube :

TABLE 7.

Amount of Dilute Standard Added	Standard Nitrate
c.c.	milligram
0	0.000
1.0	0.001
3.0	0.003
5.0	0.005
7.0	0.007
10.0	0.010
15.0	0.015
20.0	0.020
25.0	0.025
30.0	0.030
35.0	0.035
40.0	0.040

Compare the sample treated as above described with these standards by looking down vertically through the tubes at a white surface so placed in front of a window that it will reflect the light upward through them.

If the figures obtained by this comparison be divided by the number of c.c. of the samples which were evaporated, the quotient gives the number of parts per million of nitrogen in the form of nitrate.

*Reduction Method for Nitrates.*<sup>47</sup>

*Reagents.*—1. Sodium or potassium hydrate solution. Dissolve 250 grams of the purest hydrate obtainable in 1.25 liters of distilled water and boil down to one liter.

2. Aluminum foil. Use strips about 5 cm. long, .012 mm. thick, and of such a width that each strip weighs about 0.35 gram.

*Procedure.*—Put 50 c.c. of the sample, or a smaller portion diluted to 50 c.c., in a test tube about 30 cm. long and 15 mm. in diameter. Add five c.c. of the sodium (or potassium) hydrate solution and a strip of the aluminum foil. Place a loose stopper in the mouth of the tube and let stand at room temperature over night. After the reduction is complete put the contents of the tube in a Kjeldahl distilling flask and distill with steam. Cool the distillates and nesslerize in the usual way. If the sample is high in nitrate, dilute with ammonia-free water an aliquot portion of the supernatant liquid in the reduction tube and nesslerize directly.

In this process it is necessary in all cases to correct for free ammonia and nitrites, and where the free ammonia is very high it shall be boiled off before the determination of nitrates is made, restoring to the boiled sample its original volume by adding ammonia-free water.

A control determination, using the same batch of reagents and nitrogen-free water, shall always be made, because the accuracy of the procedures depends largely upon the purity of the reagents used.

#### TOTAL NITROGEN.

In sewage work it is frequently of assistance to record and study the total nitrogen contents. This is ordinarily done by adding together the nitrogen in the form of organic nitrogen, free ammonia, nitrites and nitrates, each of which is determined as already described.

Sometimes it is desirable or convenient to determine the total nitrogen by a single determination as follows:

Digest the sample in the same manner as directed for the determination of organic nitrogen, stopping the procedure just before the addition of the permanganate. From this point proceed as directed by the Association of Official Agricultural Chemists<sup>48</sup> for the determination of total nitrogen in fertilizers containing nitrates.

## DETERMINATION OF RESIDUE ON EVAPORATION.

TOTAL RESIDUE, OR TOTAL SOLIDS.<sup>49</sup>

*Procedure.*—Ignite and weigh a clean platinum dish, and into it measure 100 c.c. of the water. If the water is of high magnesium content, add 25 c.c. of  $\frac{N}{50}$  sodium carbonate solution to the water, correcting for this addition in the computation. Evaporate to dryness on a water bath. Then heat the dish in an oven (surrounded by boiling glycerin solution or toluene) at a temperature of about 103° C. for one-half hour. Then let it remain in a desiccator over sulphuric acid until cool, and weigh. The increase in weight gives the total solids or residue on evaporation.

This residue in the case of sewages and waters high in organic matter is generally ignited to burn off the organic matter which, with some volatile matters, constitutes the "loss on ignition."

With waters low in organic matter but relatively high in iron, this residue is frequently used as a matter of convenience for the determination of iron.

LOSS ON IGNITION AND FIXED RESIDUE.<sup>50</sup>

Heat the platinum dish containing the residue in a "radiator" which consists of another platinum dish large enough to allow an air space of about half an inch between the inner and outer dishes, the inner dish being supported by a triangle of platinum wire laid on the bottom of the outer dish. Over the inner dish is suspended a disc of platinum foil large enough to cover the outer dish, to radiate the heat into it. The larger dish is heated to bright redness until the residue is white or nearly so. Allow the dish to cool, and moisten the residue with a few drops of distilled water; dry the residue in an oven for half an hour, cool in a desiccator and weigh. This weight gives the fixed solids or fixed residue on evaporation, and the difference between it and the total solids, or the total residue on evaporation, gives the loss on ignition.

The manner in which the residue behaves as to odor and color upon ignition in some cases gives a helpful clue to the character of the organic matter, and in such instances the changes shall be made a matter of record.



## SUSPENDED MATTER, OR SUSPENDED SOLIDS.

This determination is made by obtaining the difference between the total solids in the unfiltered portion of a sample and in a portion from which the suspended matters have been removed. For sewages and waters containing suspended matter not too small in size, filtration may be done through a filter paper. For clay-bearing waters, suspended matter is best removed by a Berkefeld filter. Do not use a Pasteur filter. The use of a tarred filter of asbestos in a Gooch crucible<sup>50a</sup> may be found advantageous, and the suspended matter determined directly.

Treat unfiltered and filtered samples alike as regards the addition or omission of sodium carbonate solution.

The volume<sup>51</sup> of suspended matter in connection with sewage work in England has received considerable attention. In America the subject has not been studied much as yet.

## LOSS ON IGNITION AND FIXED RESIDUE DUE TO SUSPENDED AND DISSOLVED MATTERS.

By treating the total residue from the filtered sample in the same manner as above described for the total residue, there is obtained the loss on ignition due to dissolved matters, and by difference the loss on ignition due to suspended matters.

DETERMINATION OF IRON.<sup>52</sup>

Iron is found in water in dissolved and suspended forms and in both ferrous and ferric conditions, depending upon the nature of the sample. In ground waters the iron is frequently in an unoxidized and soluble condition, partly in combination with organic matter and partly as a carbonate or perhaps sulphate. Silt-bearing waters contain much iron in suspension and in an oxidized form. Sewages and sewage effluents, particularly those associated with manufacturing wastes, contain various forms and combinations of iron of different degrees of solubility and oxidation.

TOTAL IRON.<sup>53</sup>

*Reagents.*—1. Standard iron solution. Dissolve 0.7 gram of crystallized ferrous ammonium sulphate in 50 c.c. of distilled water and add 20 c.c. of dilute sulphuric acid. Warm the solution slightly and add potassium permanganate until the iron is



completely oxidized. Dilute the solution to one liter. One c.c. of the standard solution equals 0.0001 gram Fe.

2. Potassium sulphocyanide solution. Dissolve 20 grams of the salt in one liter of distilled water.

3. Dilute hydrochloric acid. One volume of acid (Sp.gr. 1.2) and one volume of distilled water. This shall be free from nitric acid.

4. Potassium permanganate solution  $\frac{N}{5}$  6.30 grams per liter.

*Procedure.*—Evaporate 100 c.c. of the sample to dryness, or use the residue left after the determination of solids, as previously described. With silt-bearing waters the quantity of iron is sometimes so great that it is necessary to use as little as 10 c.c. of the sample. With such waters evaporation should be made in the presence of 5 to 10 c.c. of strong hydrochloric acid to effect complete solution of the iron. If the sample of water contains much organic matter, destroy this by ignition, taking care not to prolong the ignition so as to render the iron too difficultly soluble.

Cool the dish and add five c.c. of dilute hydrochloric acid to moisten the whole of the inner surface of the dish. Place the dish on the steam bath for two or three minutes and again moisten the whole inner surface of the dish by allowing the hot acid to flow over it. Add 5 to 10 c.c. of distilled water to rinse down the sides of the dish, and again place on the water bath for about three minutes.

The hot acid solution is washed from the dish with distilled water into a 100 c.c. nessler tube. Filter the sample if necessary, carefully washing the filter paper with hot water. Add a drop or two of potassium permanganate solution to oxidize the iron to a ferric condition. The color of the permanganate should persist for at least five minutes; if not, add more permanganate solution, a drop at a time.

To the cooled solution 10 c.c. of potassium sulphocyanide solution are added, and the volume made up to 100 c.c. and well mixed.

Immediately compare the resulting color with that in permanent standards (see below) or in a series of standards prepared side by side with the sample in 100 c.c. nessler tubes in which amounts

of standard iron solution ranging from 0.05 to 4 c.c. are first diluted with water to about 50 c.c. Five c.c. of dilute hydrochloric acid and a drop or two of potassium permanganate are added to each tube of standard solution, and all are diluted to 100 c.c. The number of standards needed is governed by the quantity of iron likely to be present in the sample examined.

Potassium sulphocyanide shall be added to each of the standard solutions at the same time that this reagent is added to the samples of water under examination. Comparison of the sample with the standards, which are made up to 100 c.c. after adding the sulphocyanide and mixing, should be made immediately.

PERMANENT IRON STANDARDS.<sup>35</sup>

*Reagents.*—1. Platinum solution. Dissolve 2 grams of potassium platinic chloride in distilled water, add 100 c.c. of strong hydrochloric acid and make up to one liter with distilled water.

2. Cobalt solution. Dissolve 24 grams of dry cobaltous chloride crystals in a small amount of distilled water, add 100 c.c. of strong hydrochloric acid, and make up to one liter with distilled water.

*Procedure.*—Prepare a series of standards by putting the following amounts of the platinum and cobalt solutions in 100 c.c. nessler tubes and making up to 100 c.c. with distilled water.

TABLE 8.

No. of c.c. Standard Iron Solution	No. of c.c. Platinum Solu- tion	No. of c.c. Cobalt Solution
.0	0	0
.1	2	1.0
.3	6	3.0
.5	10	5.0
.7	14	7.5
1.0	20	11.0
1.5	28	17.0
2.0	35	24.0
2.5	39	32.0
3.0	40	43.0
3.5	40	55.0

Take one of the treated samples, add 10 c.c. of potassium sulphocyanide, mix, and compare *immediately* with the permanent standards. Then proceed in a like manner with the other samples.

## TOTAL IRON IN SOLUTION.

Determine, by the same method as above given for total iron, the iron in the sample after filtration. Do not forget that oxidation during filtration may precipitate ferrous iron in some samples. Generally this is not the case.

## TOTAL IRON IN SUSPENSION.

This may be determined by taking the difference between the total iron obtained by the above method in the unfiltered sample and the dissolved iron found by the same method in the filtered sample.

FERROUS IRON.<sup>54</sup>

The total ferrous iron shall be determined from an unfiltered sample, and the dissolved ferrous iron from a sample of water which has been freed from matters in suspension.

*Reagents.*—1. Standard iron solution. Dissolve 0.7 gram of crystallized ferrous ammonium sulphate in one liter of water to which are added 10 c.c. of dilute sulphuric acid. This solution easily oxidizes, and should be freshly prepared when needed. One c.c. of this standard solution contains 0.0001 gram of Fe.

2. Potassium ferricyanide solution. Dissolve five grams of the salt in one liter of distilled water. Use a freshly prepared solution.

3. Dilute sulphuric acid. Dilute one part of sulphuric acid, specific gravity 1.84, with five parts of distilled water.

*Procedure.*—To 50 c.c. of the sample add 10 c.c. of dilute sulphuric acid and 15 c.c. of potassium ferricyanide solution. The whole is made up to 100 c.c. with distilled water. Before the cyanide solution is added, the suspended matter is removed by filtration if necessary. The color obtained in the sample in the above treatment is compared with standards made from the ferrous iron solution as follows:

Place in 100 c.c. nessler tubes, in the following order, 75 c.c. distilled water, 10 c.c. dilute sulphuric acid, and 15 c.c. potassium ferricyanide solution, and mix well the contents of the tube. Prepare as many tubes in this way as are desired in order to obtain standards comparable to the iron contents of the samples being examined. Add various quantities of standard ferrous

iron solution to several tubes, mix well, and compare the resulting colors with the samples *immediately*.

#### FERRIC IRON.

The amount of ferric iron both in solution and in suspension is determined by the difference between the total iron and the ferrous iron obtained by the methods already described.

#### VOLUMETRIC METHOD FOR SAMPLES WITH HIGH IRON CONTENTS.<sup>55</sup>

Some samples of sewages and waters mixed with trade wastes and mine drainage contain so much iron that it is preferable to use the volumetric method given below for the determination of both total and dissolved iron, rather than to work with such small quantities as would permit the application of the colorimetric methods above described.

When iron is present in large quantities in suspension, as in some sewages and septic tank effluents, it may be filtered off and the residue washed, ignited, and fused with potassium and sodium carbonate. The fusion is then extracted with hydrochloric acid.

After the iron is in solution it is reduced with zinc and titrated directly with  $\frac{N}{10}$  potassium permanganate solution. The effect of free hydrochloric acid on the free permanganate solution may be avoided by adding manganous sulphate to the solution.

The quantity of iron present may be readily computed from the number of c.c. of  $\frac{N}{10}$  potassium permanganate used, each c.c. of which corresponds to 0.0056 gram of iron (Fe).

#### SEPARATION AND DETERMINATION OF LEAD, ZINC, COPPER, AND TIN.<sup>56</sup>

Lead, zinc, copper, and tin determinations are of importance in connection with the solvent action of some waters upon pipes and other containers. The use of certain "germicides" also involves some of these tests.

The first three may be determined (a) colorimetrically or (b) electrolytically. The former method is not so accurate as a combination of both, and is chiefly of value as a qualitative test.

It is possible to make a rough estimation of the amount of lead present in clear waters by acidifying with acetic acid, saturating with hydrogen sulphide and comparing the color produced with



that produced by standard lead solutions contained in nessler tubes similar to those for containing the sample. This method, however, is not applicable when the water is colored or contains iron, and in those cases the following method is advised:

*Reagents.*—1. Standard lead solution. To a strong solution of lead acetate add a slight excess of sulphuric acid, filter off and wash the precipitate. Dissolve it in strong ammonium acetate solution. Make up to a known volume and determine the lead in an aliquot part by precipitating with potassium bichromate and weighing the lead chromate. Dilute an aliquot part so that one c.c. of the dilute solution equals 0.001 gram of lead.

2. Ammonium chloride, 25 per cent solution.
3. Ammonia water, specific gravity 0.96.
4. Hydrogen sulphide water.
5. Sulphuric acid, Sp. gr. 1.84.
6. Potassium oxalate crystals.
7. Potassium sulphate crystals.
8. Ammonium acetate, strong solution.
9. Acetic acid, 50 per cent.
10. Alcohol, 95 per cent.
11. Alcohol, 50 per cent.
12. Nitric acid, 1 to 10, and strong acid (Sp. gr. 1.42).
13. Hydrochloric acid, Sp. gr. 1.20.
14. Urea crystals.

#### PROCEDURE FOR LEAD, ZINC, AND COPPER.

Concentrate (§ 1)\* rapidly by boiling in a seven-inch porcelain dish, over a free flame, three or four liters of the sample to be tested—or more if very small amounts of the metals are suspected—to a volume of about 30 c.c. Add 10 or 15 c.c. ammonium chloride reagent to assist in the separation of the sulphides, then add a few drops of strong ammonia and a considerable excess of saturated hydrogen sulphide water. After standing some time, preferably over night, add a little more ammonia and hydrogen sulphide water; boil the contents of the dish a few moments and filter.

\* The section numbers in this text description refer to the schematic tables at the end.



The precipitate (§ 2) contains all the lead, zinc, copper and iron as sulphides; also the suspended organic matter. The soluble coloring matter passes into the filtrate (§ 3).

Wash the precipitate a few times with hot water and return the precipitate on the filter to the original dish and boil with dilute nitric acid (1 part acid Sp. gr. 1.42 to 10 parts water), rubbing the sides of the dish with a bit of filter paper, if necessary, to detach any sulphide precipitate which adheres. After filtering, and washing several times with hot water, evaporate the filtrate and washings in the original dish to a bulk of about 10 or 15 c.c., cool, add five c.c. concentrated sulphuric acid, and heat over a lamp until copious fumes of sulphuric acid appear.

At this point if lead and copper are known to be absent and zinc alone is to be determined (§ 13), dilute the contents of the dish slightly; add an excess of ammonia to precipitate iron, and filter. Make the filtrate slightly acid with sulphuric acid; concentrate to a volume of about 150 c.c. and transfer to a weighed platinum dish; add two grams of pure potassium oxalate and 1.5 grams of pure potassium sulphate. Place the dish in circuit on an electrolytic apparatus with a current of about 0.3 ampere, and allow it to remain for three hours.

Siphon off the solution in the dish and at the same time run into the dish a stream of distilled water while the current is still on, in order to expel the free sulphuric acid which might dissolve some of the zinc if the current was broken at first. When the acid is expelled, break the current, remove the dish, wash with water from a wash bottle, then with 95 per cent alcohol free from residue, dry at 70° C., cool and weigh.

The difference between this weight (§ 10) and the weight of the empty platinum dish gives the amount of metallic zinc.

Some difficulty has been experienced in this determination in obtaining reagents which would give a perfect blank. In all cases blank determinations are made with each new lot of reagents and corrections made in the results when necessary.

If lead is present, however, dilute slightly (with water) the contents of the dish, after fuming with sulphuric acid, and treat with 150 c.c. of 50 per cent alcohol, in order to render the lead

sulphate insoluble. After standing some time, preferably over night, filter off the lead sulphate and wash with 50 per cent alcohol.

Dissolve the precipitate by boiling the filter containing the lead sulphate in ammonium acetate solution in a porcelain dish. (§ 4). Filter into a 100 c.c. nessler tube and wash the filter with boiling water containing a little ammonium acetate. Divide this filtrate in halves and treat one-half with saturated hydrogen sulphide water in order to get an approximation of the amount of lead present. To the other half, or an aliquot portion, if a large amount of lead is present, add a few drops of acetic acid, then an excess of saturated hydrogen sulphide water, and compare the color obtained with a set of standards made by treating with hydrogen sulphide water known amounts of standard lead solution.

To each of the standards add also a little acetic acid and ammonium acetate solution.

After thus determining the lead, if zinc is present but no copper, concentrate the filtrate from the lead sulphate to expel the alcohol, and remove the iron by an excess of ammonia. Acidify the filtrate from the iron with sulphuric acid, concentrate and electrolyze for zinc as previously described.

If copper also is present (§ 5), however, concentrate the filtrate from the lead until the alcohol is expelled and add an excess of ammonia.

§ 6. Filter off any iron precipitate.

Neutralize the filtrate (§ 7) with sulphuric acid, add 10 c.c. of concentrated sulphuric acid and one gram of urea, and electrolyze the solution for two hours with a current of about 0.5 ampere. At the end of this period break the circuit, empty the dish and wash the deposit with water, saving the filtrate and washings which contain the zinc.

Dry in a water-oven and weigh; (§ 8) then dissolve the copper off the dish with dilute nitric acid (1 to 10), wash the dish again with water, dry and weigh. The difference in weight gives the copper.

After the copper is all deposited treat the solution containing the zinc with ammonia until nearly all the sulphuric acid is

neutralized, concentrate to slightly less than the capacity of the dish and add potassium sulphate and oxalate, as previously described.

The solution is now ready to be electrolyzed for zinc. This solution, however, is generally saturated with ammonium salts due to neutralizing the large quantity of sulphuric acid, and it is frequently impossible to get the zinc deposited firmly on the dish before the salts begin to crystallize out and interfere. To avoid this difficulty half the solution (diluted so as to fill the dish) may be taken and electrolyzed for zinc, or if the amount of zinc is very low, all of the zinc may be precipitated as sulphide in acetic acid solution, ignited to oxide, and weighed. This difficulty will not occur when copper is absent, as there will not be such an excess of ammonium salts present.

#### PROCEDURE FOR COPPER ONLY.

When copper alone is to be determined (§ 11), concentrate the original water to small bulk with a little hydrochloric acid and 5 to 10 c.c. of concentrated nitric acid. When near dryness, add 10 c.c. or more of concentrated sulphuric acid, and heat over a lamp until copious fumes of sulphuric acid come off. Dilute with water, boil and filter into a beaker, washing with hot water. Neutralize the filtrate with ammonia, add a slight excess of sulphuric acid and a considerable excess of hydrogen sulphide water; heat to boiling, add more hydrogen sulphide water, and allow to stand some time, preferably over night. Filter off the sulphides. Dissolve the precipitate in dilute nitric acid; (§ 12) fume with sulphuric acid as previously described, and proceed as above described in the case of copper and zinc being present after the removal of the lead.

If zinc is present little or none of it will be precipitated as sulphide, and if any, it will not interfere in the determination of the copper.

If lead is present, practically all of it will be filtered off as sulphate after the first fuming with sulphuric acid, but as a precautionary measure allow the solution which is ready for electrolysis to stand with a slight excess of sulphuric acid present,

before adding the 10 c.c. of acid and the urea. If any precipitate forms, the solution is filtered directly into the platinum dish.

## PROCEDURE FOR TIN.

In connection with the above methods it may be remarked that small quantities of tin are occasionally met with in waters that have passed through tin or tin-lined pipes. This metal, if

TABLE 9.

SCHEME FOR THE SEPARATION OF LEAD, ZINC AND COPPER.

§ 1. Concentrate sample. Add 10 c.c.  $\text{NH}_4\text{Cl}$ , a few drops  $\text{NH}_4\text{OH}$  and saturate with  $\text{H}_2\text{S}$ . Allow to stand, add  $\text{NH}_4\text{OH}$  and  $\text{H}_2\text{S}$ . Boil, filter, and wash.

§ 2. Dissolve the precipitate in dilute  $\text{HNO}_3$ . Filter and wash. Evaporate to 10 or 15 c.c.; cool; add 5 c.c. concentrated  $\text{H}_2\text{SO}_4$  and heat until  $\text{H}_2\text{SO}_4$  is given off. Dilute slightly and treat with 150 c.c. of 50 per cent alcohol. Allow to stand; filter, and wash with 50 per cent alcohol.

§ 3. The filtrate contains the coloring matter. Reject.

§ 4. The precipitate contains the Pb. Dissolve in  $(\text{NH}_4)_2\text{AC}$  solution. Filter into a 100 c.c. nessler tube and wash with water containing  $(\text{NH}_4)_2\text{AC}$ . Divide filtrate in halves. Saturate one half with  $\text{H}_2\text{S}$ . Determine the Pb in the other half by adding  $\text{AC}$  and  $\text{H}_2\text{S}$  and then comparing with standard containing known amounts of Pb.

§ 5. The filtrate contains the Zn and Cu. Concentrate to expel alcohol. Add excess of ammonia, filter and wash precipitate.

§ 6. The precipitate contains the Fe. Reject.

§ 7. The filtrate contains the Zn and Cu. Neutralize with  $\text{H}_2\text{SO}_4$ . Add 10 c.c. concentrated  $\text{H}_2\text{SO}_4$  and 1 g. urea. Electrolyze for two hours with a current of 0.5 ampere. Break circuit. Empty dish and wash.

§ 8. The deposit is Cu. Dry and weigh as Cu.

§ 9. The solution contains the Zn. Nearly neutralize with  $\text{NH}_4\text{OH}$ . Concentrate to less than the capacity of the dish. Add 2 g.  $\text{K}_2\text{OX}$  and 1.5 grams of  $\text{K}_2\text{SO}_4$ . Electrolyze for 3 hours with a current of 0.3 ampere. Siphon off solution, break circuit, wash with water, then alcohol, dry at  $70^\circ \text{C}$ , cool and weigh.

§ 10. The weighed residue is metallic Zn.



TABLE 10.

SCHEME FOR COPPER ONLY.

§ 11. Add HCl and  $\text{HNO}_3$  and concentrate. Add 10 c.c. or more concentrated  $\text{H}_2\text{SO}_4$  and heat until  $\text{H}_2\text{SO}_4$  volatilizes. Dilute, boil, filter and neutralize with  $\text{NH}_4\text{OH}$ . Add a slight excess of  $\text{H}_2\text{SO}_4$  and a considerable excess of  $\text{H}_2\text{S}$  water. Heat to boiling, add more  $\text{H}_2\text{S}$  water and allow to stand. Filter off sulphides.

§ 12. The precipitate contains the Cu. Dissolve in dilute  $\text{HNO}_3$ , fume with  $\text{H}_2\text{SO}_4$ , precipitate iron with  $\text{NH}_4\text{OH}$ , and proceed as in Section 7.

Reject the filtrate from the sulphides.

TABLE 11.

SCHEME FOR ZINC ONLY.

§ 13. Follow scheme for all three metals as given in Table 9 through Section 5. Nearly neutralize the filtrate with  $\text{H}_2\text{SO}_4$ , concentrate to less than the capacity of the dish and electrolyze as directed in Section 9.

present, is removed with the iron by ammonia in the lead, zinc and copper separation; and in the method for copper alone, it is removed in the same way and may be further avoided by dissolving the sulphides in strong nitric acid (Sp. gr. 1.42) when any tin present will be separated as an insoluble compound.

There is as yet no satisfactory method for the quantitative separation of small quantities of tin.

These schematic tables illustrate the procedures given. The figures refer to the section numbers in the text.

DETERMINATION OF HARDNESS.<sup>57</sup>

The hardness of a water is caused by the presence of certain soluble mineral constituents which consist chiefly of the salts of calcium and magnesium. Hardness is commonly measured by the soap-destroying power of the water. The addition of a potassium or sodium soap to a hard water decomposes the soap and produces insoluble soaps of lime and magnesia. The solubility of calcium and magnesium carbonate in a water beyond certain limits depends upon the presence of carbonic acid, and results in what are called bicarbonates. As the carbonic acid is



removed by boiling the water, the normal carbonates of lime and magnesia are in consequence precipitated. The precipitation, however, is not complete, and portions of the calcium and magnesium carbonates still remain dissolved, even after prolonged boiling. To the extent to which these salts are precipitated, the hardness of the water is diminished or softened by boiling. The hardness thus removed is called "temporary hardness." The hardness which still remains after boiling the water is largely due to the sulphates and chlorides of lime and magnesia, and to the carbonates of these bases still held in solution. The hardness not removed by boiling is termed "permanent hardness."

For many years hardness determinations by the soap method have been included in the sanitary analyses of waters for the purpose of rapidly approximating the quantities of calcium and magnesium salts in the water. In more precise terms, these results record the soap-consuming properties of the water. Rapid developments in connection with the coagulation<sup>58</sup> and purification of muddy water, and developments in the field of water softening,<sup>59</sup> have combined to direct especial attention to the group of compounds associated with hardness. The more important tests for this allied group are given in this report.

#### TOTAL HARDNESS BY THE SOAP METHOD.<sup>60</sup>

*Reagents.*—1, Standard calcium chloride solution.—Dissolve 0.2 gram of pure calcite (calcium carbonate) in a little dilute hydrochloric acid, being careful to avoid loss of solution by spattering. Evaporate it to dryness several times to expel excess of acid. Dissolve in distilled water and make up to one liter. One c.c. is equivalent to 0.0002 gram of calcium carbonate.

2. Standard soap solution.—Dissolve 100 grams of dry white Castile soap in one liter of 80 per cent alcohol, and allow this solution to stand several days before standardizing. From the above stock solution dilute with 70 per cent alcohol such a quantity that the resulting diluted soap solution will give a permanent lather when 6.40 c.c. of it are properly added to 20 c.c. of standard calcium chloride solution. Usually from 75 c.c. to 100 c.c. of the stock soap solution are required for making one liter

of the standard soap solution. Pure potassium soap made from lead plaster and potassium carbonate may be used to advantage in place of Castile soap.

In standardization 20 c.c. of the calcium chloride solution are put into a 250 c.c. glass stoppered bottle and diluted to 50 c.c. with distilled water which has been recently boiled and cooled. There is then added from a burette 0.2 or 0.3 c.c. of soap solution at a time, shaking the bottle vigorously after each addition until a lather over the entire surface of the water is formed which remains continuous for five minutes after the bottle is laid upon its side. When the soap solution is of the strength above stated, then the quantity of calcium carbonate equivalent to each cubic centimeter of the soap solution is indicated in the following table:

TABLE 12

TABLE OF HARDNESS, SHOWING THE PARTS PER MILLION OF CALCIUM CARBONATE ( $\text{CaCO}_3$ ) FOR EACH TENTH OF A CUBIC CENTIMETER OF SOAP SOLUTION WHEN 50 C.C. OF THE SAMPLE ARE USED.

c.c. of Soap Solution	0.0 c.c.	0.1 c.c.	0.2 c.c.	0.3 c.c.	0.4 c.c.	0.5 c.c.	0.6 c.c.	0.7 c.c.	0.8 c.c.	0.9 c.c.
0.0 .....								0.0	1.6	3.2
1.0 .....	4.8	6.3	7.9	9.5	11.1	12.7	14.3	15.6	16.9	18.2
2.0 .....	19.5	20.8	22.1	23.4	24.7	26.0	27.3	28.6	29.9	31.2
3.0 .....	32.5	33.8	35.1	36.4	37.7	38.0	40.3	41.6	42.9	44.3
4.0 .....	45.7	47.1	48.6	50.0	51.4	52.9	54.3	55.7	57.1	58.6
5.0 .....	60.0	61.4	62.9	64.3	65.7	67.1	68.6	70.0	71.4	72.9
6.0 .....	74.3	75.7	77.1	78.6	80.0	81.4	82.9	84.3	85.7	87.1
7.0 .....	88.6	90.0	91.4	92.9	94.3	95.7	97.1	98.6	100.0	101.5

This table does not provide for the use of as large a volume of soap solution for a single determination as was formerly the case, owing to the fact that the end point becomes somewhat obscured in the presence of magnesium salts, as explained beyond, when more than about seven c.c. are used.

*Procedure.*—Measure 50 c.c. of the water into a 250 c.c. bottle and add soap solution in small quantities and in precisely the same manner as described under the standardization of the soap solution. From the result obtained there may be noted from the table above the total hardness of the water in terms of calcium carbonate.

When adding the soap solution to waters containing mag-

nesium salts, it is necessary to avoid mistaking the false or magnesium end-point for the true one.<sup>61</sup> Consequently, after the titration is apparently finished, read the burette and add about 0.5 c.c. of soap solution. If the end point was due to magnesium, the lather now disappears. Soap solution must then be added until the true end point is reached. Usually the false lather persists for less than five minutes.

When more than seven c.c. of soap solution are required for 50 c.c. of the water, it is necessary to take less of the sample and dilute to 50 c.c. with distilled water which has been recently boiled and cooled. This step reduces somewhat the disturbing influence of magnesium salts,<sup>62</sup> which consume more soap than do equivalent weights of calcium salts.

At best the soap method is not a precise test on account of the varying amounts of calcium and magnesium present in different waters. For hard waters, especially in connection with processes for purification and softening, it is advised that this method be not used.

When the same water is frequently analyzed, it may be of assistance to standardize the soap solution against a mixture of calcium and magnesium salts, the relative proportions of which approximate those found in the water.

When free carbonic acid is present in the sample in considerable amount, it should be removed by aëration.

The strength of the soap solution should be determined from time to time, to make sure that it has not materially changed while standing. Unless otherwise stated, record all results in terms of calcium carbonate.

English degrees of hardness, Clark's scale, are equivalent to grains of calcium carbonate per imperial gallon, and are multiplied by 14.3 to give parts per million.

French degrees of hardness represent parts per 100,000 of calcium carbonate, and are multiplied by 10 to give parts per million.

German degrees of hardness represent parts per 100,000 of calcium oxide, and are multiplied by 17.8 to give parts of calcium carbonate per million.

TOTAL HARDNESS BY INCRUSTANT METHOD.<sup>63</sup>

This method gives fairly satisfactory results for hard waters. It consists in adding standard sulphuric acid to the sample until the alkalinity is neutralized. See Alkalinity, p. 61. Then the incrustants (in which form all of the calcium and magnesium are present) are determined with the aid of soda reagent by the procedure detailed on p. 65.

## TOTAL HARDNESS BY COMPUTATION FROM CALCIUM AND MAGNESIUM CONTENTS.

The most accurate method of ascertaining total hardness is by computation from the results of determinations of calcium and magnesium in the water, which may be obtained by the methods noted below. The calcium should be computed as calcium carbonate, and the magnesium should also be calculated ordinarily to an equivalent quantity of calcium carbonate, although in connection with some water softening problems the magnesium is preferably calculated to magnesium carbonate.

For purposes of ordinary analysis it is not considered by the committee that it is necessary to obtain the total hardness by this means, although, if waters behave somewhat abnormally with soap solutions, as do magnesium waters, it is desirable as a control, especially in the case of waters with which the analyst is not familiar.

In connection with water softening projects, the determination of the total hardness by this method at intervals is regarded as imperative, as a control for the method above.

CALCIUM BY THE GRAVIMETRIC METHOD.<sup>64</sup>

After the removal of silica, iron and alumina from a known volume of the water, add to the filtrate an excess of ammonium oxalate, filter off the calcium oxalate, ignite and weigh as calcium oxide in accordance with regular procedures for calcium determinations, and compute the result in parts of Ca per million.

CALCIUM BY THE VOLUMETRIC METHOD.<sup>65</sup>

Calcium may be determined volumetrically with equally satisfactory results and somewhat more quickly by washing the precipitate of calcium oxalate obtained as before with as little hot water as possible, and then dissolving it in dilute sulphuric acid and titrating with a standard solution of potassium permanganate.



MAGNESIUM BY THE GRAVIMETRIC METHOD.<sup>64</sup>

Concentrate the filtrate from which the calcium has been removed; add sodium phosphate and ammonia, and allow the solution to stand for 12 hours in a cool place; then filter, ignite the precipitate and weigh as magnesium pyrophosphate, and compute the results in parts of Mg per million.

Magnesium in the filtrate from the calcium oxalate precipitate may also be determined by turbidimetric<sup>66</sup> comparison with standards of magnesium sulphate.

MAGNESIUM BY THE VOLUMETRIC METHOD.<sup>67</sup>

Measure 100 c.c. of the water into a six-inch porcelain dish. Add exactly enough  $\frac{N}{50}$  sulphuric acid solution to make the water neutral to lacmoid or erythrosine, as found by the determination of the alkalinity (see page 61). Boil down to a volume of about 30 or 40 c.c. to expel the free, half-bound and bound carbonic acid and to concentrate the solution.

Introduce by means of a pipette 25 c.c. of a clear saturated solution of lime water of known strength into a 150 c.c. glass stoppered flask. While still hot, transfer immediately to this flask the water concentrated as above described. Rinse out the porcelain dish with hot boiled distilled water and make up the solution to about two c.c. above the 150 c.c. line in the flask. Mix well, stopper immediately and cool.

When the precipitated magnesium hydrate has settled completely, pipette off very carefully, in order not to disturb the precipitate at the bottom of the flask. 50 c.c. of the clear solution. Run this solution into a known quantity of  $\frac{N}{50}$  sulphuric acid, which is not quite sufficient to neutralize it. Finish the titration by adding more acid, using phenolphthalein as an indicator.

If C represents the number of c.c. of  $\frac{N}{50}$  sulphuric acid required to neutralize 25 c.c. of the lime water, and N the number of c.c. of the same acid required to neutralize the excess of lime water in the sample, then Mg in parts per million equals  $2.4 (C-3N)$ .

It is to be borne in mind that the strength of lime water solutions varies somewhat with the temperature. Where especially accurate data are required a control determination should be



made by treating the specified quantity of lime water with distilled water in the same manner as in the regular procedure.

MAGNESIUM BY THE SOAP METHOD AFTER REMOVAL OF CALCIUM.<sup>68</sup>

Approximate determinations of magnesium salts present may be obtained by shaking up about 0.1 gram of solid ammonium oxalate with 100 c.c. of the water to be examined. Then put the whole on a water bath; cool and filter through a dry filter paper; discard the first 25 c.c. of the filtrate and then titrate 50 c.c. of the remaining filtrate with soap solution in the usual way.

The amount of magnesium (Mg) is computed as 24 per cent of the hardness indicated in terms of calcium carbonate by Table 12.

The results may be made of somewhat greater accuracy if the soap solution is standardized against a solution of magnesium sulphate of known strength. In view of the different quantities of soap consumed by equivalent amounts of calcium and magnesium, this step is recommended when a special study is being made of a particular water.

TEMPORARY HARDNESS BY THE SOAP METHOD.

Temporary hardness by the soap method is sometimes estimated by the difference between the total hardness obtained as above described and the permanent hardness obtained by applying the soap test to the water after boiling. This method is not advised.

A known volume of the water is boiled gently for half an hour and the water is then allowed to cool. Recently boiled and cooled distilled water is then added so as to restore the water to its original volume. Volumetric flasks may be used for this purpose. The water is then filtered and the hardness of the filtrate determined by the soap method in the usual manner.

TEMPORARY HARDNESS BY TITRATION WITH ACID.

This is by far the most accurate method of determining the true temporary hardness, or the bicarbonate alkalinity, of all ordinary waters. Alkalinity by the method described below is determined in the original sample of water and also in the water after boiling and filtering in the manner stated in the last paragraph. The difference between the two, if any, is the correct temporary hardness.

For those ground waters containing much iron in the form of bicarbonates, the iron in this form is included as a part of the temporary hardness.

ALKALINITY.<sup>69</sup>

The alkalinity of natural waters ordinarily includes the carbonates and bicarbonates of calcium and magnesium. In some waters in the West it also includes the carbonates of sodium or potassium. Waters that are being softened contain at certain stages alkalinity due to calcium hydrate (lime water), and also sodium carbonate (soda ash).

*Procedure with Lacmoid.*—Measure 100 c.c. of the water into a porcelain evaporating dish and add 0.5 c.c. of lacmoid solution (two grams in one liter of 50 per cent alcohol). Add  $\frac{N}{50}$  sulphuric acid to the water from a burette until within one or two c.c. of the amounts necessary for neutralization have been added. The dish is then placed on a tripod and the contents heated until bubbles of steam begin to break at the surface of the water. The dish is then removed and the titration continued until a drop of the acid striking the surface of the liquid and sinking to the bottom of the dish produces no change in the uniform reddish or purple color of the solution.

The number of c.c. of  $\frac{N}{50}$  sulphuric acid used when multiplied by ten gives the number of parts per million of alkalinity in terms of calcium carbonate.

*Procedure with Erythrosine.*—Where it is desired not to use heat or where heat is not available, satisfactory results may be obtained by measuring 100 c.c. of the water to be examined into a 250 c.c. white glass stoppered bottle with 2.5 c.c. of erythrosine solution (0.1 gram of the sodium salt in one liter of distilled water) and 5 c.c. of chloroform (neutral to erythrosine). Sulphuric acid ( $\frac{N}{50}$ ) is added in small quantities, shaking the bottle vigorously after each addition of the acid. The rose color gradually disappears and is finally entirely discharged by a drop or two of the acid. A white paper held back of the bottle facilitates the detection of any trace of color remaining as the end point is approached. The calculation is the same as with lacmoid.

*Notes.*—Phenacetolin and rosolic acid behave in a manner similar to lacmoid.

Methyl orange is also used by some analysts in determining alkalinity. With this indicator it is unnecessary to heat the water to drive off free carbonic acid, such as is the case with lacmoid, phenacetolin and rosolic acid. In this regard methyl orange behaves like erythrosine. Many unsatisfactory experiences have been encountered with the use of methyl orange owing to the difficulty with which satisfactory brands of the product may be obtained, and further owing to complications when it is used with waters which are highly turbid or highly colored. Another disadvantage of its use in laboratories connected with water purification plants where coagulants are used is that it is not a "reversible" indicator, that is, it does not react acid to sulphate of alumina or sulphate of iron, as is true of lacmoid. The use of methyl orange is not recommended by the committee, although it is recognized that it is possible with many waters to get accurate alkalinity results from its use in skilled hands.

*Alkalinity due to Normal Carbonates.*<sup>70</sup>

Normal carbonates of the alkaline earth metals (or the alkalies) are present in a water when the phenolphthalein alkalinity (determined in the cold) is equal to or less than one-half of the alkalinity by lacmoid or erythrosine.

When phenolphthalein is used as an indicator in the cold, one-half of the normal carbonate alkalinity is indicated upon titration with standard sulphuric acid. Only one-half of the quantity present is indicated, because sulphuric acid causes the liberation of an equivalent amount of carbonic acid, which reacts acid to this indicator.

When phenolphthalein gives no color in the cold with a water which is alkaline to lacmoid or erythrosine, it shows that no normal carbonates are present, and that the alkalinity is wholly due to bicarbonates. See Carbonic Acid, page 73.

When the phenolphthalein alkalinity is equal to just one-half the alkalinity by lacmoid or erythrosine, it shows that all of the alkalinity is present in the form of normal carbonates.

When a water has been treated with an excess of lime water only small quantities of normal carbonates remain. The latter are measured by subtracting the phenolphthalein alkalinity from the alkalinity by lacmoid or erythrosine and multiplying the remainder by two.

*Alkalinity due to Sodium or Potassium Carbonates.*

Waters from alkaline regions which contain sodium or potassium carbonate are found to contain all of their calcium and magnesium as carbonates or bicarbonates. That is, they possess either no incrustants (sulphates, nitrates or chlorides of calcium and magnesium) or only traces of them.

The most accurate method is to determine the total alkalinity by titration with  $\frac{N}{50}$  sulphuric acid, using erythrosine or lacmoid as an indicator; then determine the calcium and magnesium contents; and subtract from the total alkalinity the computed alkalinity due to the calcium and magnesium expressed in terms of calcium carbonate. The remainder is the alkalinity due to carbonates of sodium and potassium.

This determination may also be made by applying the method, described beyond, for "incrustants with soda reagent" and noting the excess of acid required to neutralize the alkaline carbonates originally present.

With present information as to solubilities of the normal carbonates of calcium and magnesium, it is difficult in their presence to measure slight quantities of carbonates of sodium or potassium.

*Alkalinity due to Hydrates (Caustic Alkalinity).<sup>70</sup>*

When waters contain calcium or other alkaline hydrate as well as normal alkaline carbonates, the phenolphthalein alkalinity is more than one-half of that with lacmoid or erythrosine.

Determine the alkalinity with phenolphthalein in the cold, and the total alkalinity with erythrosine as an indicator. Do not use lacmoid as the heat required modifies the reactions.

The alkalinity by the former indicator subtracted from that by the latter and multiplied by two gives the normal carbonate alkalinity; this subtracted from the total alkalinity gives the hydrate (or caustic) alkalinity.

When the phenolphthalein alkalinity is less than one-half of that by lacmoid or erythrosine, then no caustic alkalinity is present—the alkalinity being due to carbonates and bicarbonates. See Carbonic Acid, page 73.

When the phenolphthalein alkalinity is the same as that by lacmoid or erythrosine, then all of the alkalinity is due to caustic alkali.



*Silver Nitrate Test for Caustic Alkalinity.*<sup>11</sup>

In the operation of water softening plants use is sometimes made of a dilute silver nitrate solution in testing the water for the presence of caustic alkalinity (lime water). A positive result is shown by the appearance of a grayish-brown color.

The evidence before the committee indicates that a positive result may not be obtained until the alkalinity in the form of a hydrate is present to the extent of about 10 parts per million. For this reason, and on account of the color complications arising when chlorides are present in considerable quantities, the use of this test is not advised where accurate data are desired.

## PERMANENT HARDNESS BY THE SOAP METHOD.

Reference has already been made to this determination in connection with the temporary hardness above described. The soap-consuming properties of a water which has been thoroughly boiled and filtered, while indicating what is properly called "permanent hardness," does not have much practical significance because it includes in addition to the sulphates and chlorides of calcium and magnesium, also the carbonates of these bases, which are naturally soluble in a water free from carbonic acid. The practice of this method and the use of the expression "permanent hardness" obtained by the soap method are so unsatisfactory that it is advised they be discontinued.

In this connection it is to be pointed out that incrustants do not occur, except perhaps as slight traces, in natural waters containing carbonates of sodium and potassium, although such waters would show a permanent hardness due to normal carbonates of calcium and magnesium.

## INCRUSTANTS BY THE SOAP METHOD.

By incrustants is meant, generally speaking, the sulphates, chlorides and nitrates of calcium and magnesium. They may be determined, for waters which are soft or moderately hard, in apparently a fairly satisfactory manner by deducting the total alkalinity from the total hardness by the soap method.

With waters which are quite hard, particularly such as contain considerable quantities of magnesium, this method is not advised.



INCRUSTANTS BY TITRATION WITH EXCESS OF SODIUM CARBONATE<sup>63</sup>

This method consists in evaporating to dryness in a platinum dish 100 c.c. of the water together with 50 c.c. of an  $\frac{N}{25}$  solution of sodium carbonate. The residue is heated over a flame at a temperature of red heat for five minutes, when the dish is cooled and the residue treated with boiled distilled water to make up the volume to 100 c.c. and allowed to settle; pipette off 25 c.c. of the clear supernatant liquid and determine the excess of sodium carbonate with  $\frac{N}{50}$  sulphuric acid, using erythrosine as an indicator.

This method has been given a thorough trial at numerous places in this country, and the consensus of opinion is that the results are quite uniformly too low on account of the solubility of the carbonates of calcium and magnesium. By the use of suitable correction factors, approximate results can probably be obtained with hard waters, but the use of the method according to the evidence now available is not advised.

INCRUSTANTS BY TITRATION WITH EXCESS OF "SODA REAGENT."<sup>63</sup>

The substitution for sodium carbonate in the method last described of the "soda reagent" appears to be a step in the right direction in determining incrusting constituents in hard waters, as it makes less soluble the salts of calcium and magnesium. It does not correct entirely, however, the error due to solubility, and it is advised that, in connection with important work in water softening, gravimetric determinations be made to ascertain the quantities of calcium and magnesium which go into solution and for which a correction factor should be applied under the regular conditions of practice, as follows:

*Procedure.*—Measure 200 c.c. of the water into a Jena glass Erlenmeyer flask; boil 10 minutes to expel free carbonic acid, and add 25 c.c.  $\frac{N}{10}$  "soda reagent" (equal parts of NaOH and  $\text{Na}_2\text{CO}_3$ ). Boil to a volume of 100 c.c. cool, rinse into a 200 c.c. graduated flask and make up to 200 c.c. with boiled distilled water. Filter, rejecting the first 50 c.c. and titrate 100 c.c. of the filtrate for excess of soda reagent, using  $\frac{N}{20}$  sulphuric acid and erythrosine as an indicator.

If  $S$  = c.c.  $\frac{N}{20}$   $\text{H}_2\text{SO}_4$  equivalent to soda reagent used, and  $N$  = c.c.  $\frac{N}{20}$   $\text{H}_2\text{SO}_4$  required for the excess (back titration), then the

incrustants in parts per million of calcium carbonate equal 12.5 (S-2N).

Water containing carbonates of sodium and potassium will require a larger amount of acid to neutralize the sample after it has been treated than is required to neutralize the volume of soda reagent originally added.

Where the total hardness is determined by this method add to 200 c.c. of the sample a sufficient quantity of  $\frac{N}{50}$  sulphuric acid to neutralize the alkalinity in accordance with the result of the latter determination (see page 61). The calcium and magnesium present in the sample as carbonates and bicarbonates are thus converted into sulphates, and the incrustants correspond to the total hardness. Boil down to 100 c.c., add 25 c.c.  $\frac{N}{10}$  soda reagent and again boil down to 100 c.c. Cool, rinse into a 200 c.c. flask and make up to 200 c.c. with freshly boiled distilled water. Titrate an aliquot portion and compute as above described the total hardness. The incrustants are of course obtained by subtracting the alkalinity from the total hardness.

Unless a water is very hard, it seems best, in using the "soda reagent" method, to obtain the incrustants by difference, as explained in the last paragraph rather than directly. The percentage error due to solubility is thus reduced.

#### DETERMINATION OF CHLORINE.<sup>72</sup>

Chlorine in waters and sewages has its origin for the most part in the common salt, which comes, generally speaking, from mineral deposits in the earth, from the ocean vapors carried inland by the wind, or from polluting materials like sewage and trade wastes, both of which contain the salt used in the household and in manufacturing. Comparison of the chlorine contents of a water with that of other waters in the general vicinity known to be unpolluted frequently affords useful information as to its sanitary quality.

*Reagents.*—1. Standard salt solution. Dissolve 16.48 grams of fused sodium chloride in one liter of distilled water. Dilute 100 c.c. of this stock solution to one liter in order to obtain a standard solution each c.c. of which contains .001 gram of chlorine.

2. Silver nitrate standard. Dissolve about 2.40 grams of silver nitrate crystals in one liter of distilled water. One c.c. of this will contain approximately .0005 gram of chlorine. Standardize this against the standard salt solution.

3. Potassium chromate. Dissolve 50 grams of neutral potassium chromate in a little distilled water. Add enough silver nitrate to produce a slight red precipitate. Filter and make up the filtrate to one liter with distilled water.

4. Aluminum hydrate. Electrolyze ammonia-free water, using aluminum electrodes. Wash the precipitate formed until free from chlorine, ammonia and nitrites. Or, dissolve 125 grams of potash or ammonia alum in one liter of distilled water. Precipitate the aluminum hydrate by cautiously adding ammonium hydrate. Wash the precipitate in a large jar by the successive addition of distilled water and by decantation until free from chlorine, nitrites and ammonia.

*Procedure.*—Use 50 c.c. of the sample in a white six-inch porcelain evaporating dish for this determination where the chlorine content is not extremely low or very high. If the chlorine is very high in amount, use 25 c.c. or even a smaller quantity if desired, diluting the volume taken with distilled water to 50 c.c. When the sample is very low in its chlorine content, more accurate results may be obtained by using 50 c.c. of the sample and adding, prior to titration, one c.c. of standard salt solution.

Some analysts prefer in testing samples low in chlorine content to concentrate the sample by evaporation. Where evaporation is resorted to care should be taken to avoid the loss of hydrochloric acid by the decomposition of magnesium chloride. This can be accomplished by the addition of a little sodium carbonate, 0.002 gram ordinarily being sufficient.

Chlorine is determined by some observers by extracting with hot distilled water the residue contained in the platinum dish in the determination of the residue on evaporation and proceeding as above described. This is permissible provided care is taken to add sodium carbonate as above indicated to guard against the loss of hydrochloric acid through the decomposition of magnesium chloride.



If the sample has a color greater than about 30 it shall be decolorized by heating it to the boiling point with washed aluminum hydrate (3 c.c. to 500 c.c. of the sample). Make the determination on the portion of the clarified sample, filtered if necessary.

If the sample is acid, neutralize with sodium carbonate; if alkaline due to hydrates, add dilute sulphuric acid until the cold liquid will just discharge the color of phenolphthalein.

Rotate the liquid in the dish to make sure that no portion of the residue on the side walls of the dish remains undissolved; if necessary, use a rubber tipped glass rod to assist in this process.

Add one c.c. of the potassium chromate solution as an indicator. Titrate with the silver nitrate solution, under similar conditions, as to volume, light and temperature as were used in standardizing the silver nitrate. The detection of the end-point is facilitated by frequent comparison of the contents of the porcelain dish in which the determination is being made with those of another dish placed alongside and containing the same quantity of chromate solution in 50 c.c. of distilled water. Where especially accurate work is desired, some analysts prefer to make these observations in a dark room provided with a yellow light.

More accurate results can be obtained for the error due to variations in the volume of the liquid and precipitate, according to the following formula:<sup>73</sup>  $X = .003V + .02$ , where  $X$  = the correction in c.c. of silver nitrate solution and  $V$  = c.c. of liquid at the end of the titration. Also, if necessary, make corrections<sup>74</sup> or modifications in treatment for the presence of sulphides and sulphocyanates.

Titration may be made by using 50 c.c. or 100 c.c. samples in nessler tubes,<sup>75</sup> provided the solutions are standardized under the same conditions.

#### DETERMINATION OF SULPHURIC ACID.

In some special problems it is desirable to know the quantity of sulphuric acid present in the water. Generally speaking, however, experience during the past decade in this country with this determination has indicated that the value of the results obtained



is not ordinarily commensurate with the labor involved. The reason of this is that it gives only the total quantity present and does not separate the sulphuric acid present as free acid or as the sulphates of the alkaline earth bases or the sulphates of sodium and potassium, or the sulphates of aluminum and iron. These determinations from a practical point of view are best attacked along the lines of acidimetry, incrustants and special tests for sulphates of iron and alumina as described elsewhere in this report.

GRAVIMETRIC METHOD.<sup>76</sup>

Where very accurate results are desired this method is to be preferred, especially for waters low in sulphates. Measure a certain volume of water into a porcelain evaporating dish, the quantity of water depending somewhat upon the probable quantity of sulphuric acid present. Add a slight excess of hydrochloric acid and evaporate to a volume of 250 c.c. if more than this is used. If silica or iron is present, or if the concentrate is not clear, add ammonia to a slight excess, boil and filter. If the sample of water is previously filtered through a Berkefeld filter tube, the precipitation with ammonia may often be omitted. Sulphuric acid is determined by precipitation with barium chloride in the usual way and the sulphuric acid computed from the weight of barium sulphate and expressed in parts of  $\text{SO}_4$  per million.

VOLUMETRIC METHOD.<sup>77</sup>

This method is most applicable to waters fairly high in sulphates. It consists in adding an excess of barium chloride to the water, precipitating the excess of barium with a known excess of neutral potassium ammonium chromate, determining the excess of the latter colorimetrically by comparing the color in the sample with the standards containing known quantities of chromate solution.

*Reagents.*—Solutions ( $\frac{N}{10}$ ) of barium chloride and of potassium ammonium chromate. To prepare the latter dissolve 7.37 grams of pure potassium bichromate in distilled water, neutralize with ammonia and dilute to one liter. Prepare these solutions so that 100 c.c. of each when boiled together produce a yellow precipitate with a supernatant liquid which is colorless and free from barium.

Otherwise, adjust the chromate solution to conform to the strength of the barium chloride solution.

*Procedure.*—Boil 100 c.c. of the sample for half an hour if necessary to free it from an excess of alkaline earth carbonates, meanwhile maintaining the liquid at a constant volume with frequent additions of distilled water to prevent the precipitation of sulphates. The sample should be neutral; if not, neutralize by the addition of hydrochloric acid. Filter into a 150 c.c. volumetric flask and add 10 to 20 c.c. of barium chloride according to the amount of sulphuric acid present, and boil for a few minutes.

Add a known volume of chromate solution so that the supernatant liquid is slightly yellow with an excess of chromate. Cool, make up to 150 c.c. and filter off 100 c.c. of the contents of the flask into a nessler tube. Estimate the excess of chromate in the filtrate by comparison with a set of standards made by taking different known volumes of chromate solution and diluting to 100 c.c. portions with distilled water in nessler tubes.

Multiply the reading obtained by 1.5 and subtract the result from the volume of chromate used; and subtract the remainder from the amount of barium chloride added. This remainder equals the number of c.c. of standard barium chloride solution used, and when multiplied by 48 gives the parts per million of sulphuric acid as  $\text{SO}_4$ .

#### DETERMINATION OF ACIDITY DUE TO MINERAL ACIDS OR TO THEIR IRON OR ALUMINUM SALTS.<sup>78</sup>

In mining regions waters frequently contain high quantities not only of carbonic acid but also of sulphuric acid and various sulphates—those of iron and aluminum giving an acid reaction.

The total acidity due to free carbonic acid, free sulphuric acid and to sulphates of iron and aluminum is determined by titrating the water in the cold with standard sodium carbonate, using phenolphthalein as an indicator.

The acidity due to free sulphuric acid is determined by titrating the water in the cold with standard sodium carbonate, using methyl orange as an indicator. Some analysts prefer to treat the water with a known excess of standard sulphuric acid, boil for 20

minutes in a porcelain dish, cool and titrate as just described to note the increase in acidity.

The acidity due both to free sulphuric acid and to the sulphates of iron and alumina is determined by titrating the water in the cold with standard sodium carbonate, using erythrosine as an indicator.

By determining the amount of iron present in the sample in the ferrous and ferric condition, according to the last of the methods already described under "Iron" the acidity due to sulphate of alumina and to sulphate of iron is estimated by difference. By determining the amount of ferrous and ferric iron as already described (see pages 47 and 48) the ferrous sulphate and the ferric sulphate may be computed.

From the data obtained as above described and from the methods of computation already given, there may be computed the quantities of each kind of acidity, expressed in parts per million of free carbonic acid and of sulphate acidity in terms of  $\text{SO}_4$ ,—both for the free sulphuric acid and the sulphates of iron and aluminum.

#### DETERMINATION OF ALUM OR SULPHATE OF ALUMINA.

Where these chemicals are used for the coagulation of muddy or highly colored waters, it is essential that care be taken in operating the plant to prevent any of these undecomposed salts appearing in the water after treatment. There are two ways by which a test for their presence is made, one being the measure of acidity of the water when titrated with such indicators as lacmoid or erythrosine, and the other the so-called "logwood" test.

#### ACIDITY BY LACMOID OR ERYTHROSINE.<sup>79</sup>

This determination is made in a corresponding manner to the alkalinity determination already described on page 6. If the reaction is not alkaline, then  $\frac{N}{50}$  sodium carbonate may be used in measuring the amount of acidity due to the alum or to the sulphate of alumina, and the results expressed in terms of calcium carbonate (or computed into grains of sulphate of alumina per gallon).

Lacmoid, phenacetolin or erythrosine may be safely used as

indicators for this test. Methyl orange cannot be used, because as already explained it is not reversible, and indicates only on the alkaline side of the neutral point.

#### LOGWOOD TEST.<sup>80</sup>

This test is based upon the fact that solutions of logwood or of hematoxylin in the presence of alum and in an acid solution give a well defined reddish or brownish color, the intensity of which can be estimated by comparing the color produced by the sample with a series of standard colors prepared by treating in a corresponding manner a series of solutions containing known quantities of alum or sulphate of alumina.

This test has been widely experimented with, and while it has given satisfactory results in some hands, the consensus of opinion is that it is a difficult test to apply satisfactorily under many conditions in practice. This is due to the fact that it has to be carried out in an acid solution and the acid so supplied has a solvent action upon minute particles of colloidal aluminum hydrate (and perhaps on aluminum silicate) such as are present in very minute quantities in numerous samples of filtered or subsided water. An imperfectly clarified water may thus give erroneously a positive test for sulphate of alumina or alum.

As a means of testing samples for the presence of suspended aluminum hydrate this method is of value when the water is alkaline to lacmoid or erythrosine.

#### DETERMINATION OF CARBONIC ACID.<sup>81</sup>

Carbonic acid may exist in water in three forms, namely, as free, half-bound and bound carbonic acid. Free carbonic acid requires a separate determination, but the remaining forms in which carbonic acid is present may ordinarily be computed from the alkalinity determinations already described.

#### FREE CARBONIC ACID.

*Reagents.*—Standard  $\frac{N}{2}$  solution of sodium carbonate. Dissolve 2.41 grams of dry sodium carbonate in one liter of distilled water which has been boiled and cooled in an atmosphere free from carbonic acid. Preserve this solution in bottles of resistant



glass, protected from the air by tubes filled with soda-lime. One c.c. equals 0.001 gram of  $\text{CO}_2$ .

*Procedure.*—Measure 100 c.c. of the sample into a tall narrow vessel, preferably a 100 c.c. nessler tube, and titrate rapidly with the  $\frac{N}{22}$  sodium carbonate solution, stirring gently until a faint but permanent pink color is produced by phenolphthalein.

The number of c.c. of  $\frac{N}{22}$  sodium carbonate solution used in titrating 100 c.c. of water, multiplied by 10, gives the parts per million of free carbonic acid as  $\text{CO}_2$ .

Owing to the ease with which free carbonic acid escapes from water, particularly when present in considerable quantities, it is highly desirable that a special sample<sup>82</sup> should be collected for this determination, which should preferably be made on the ground. If the analysis cannot be made on the ground, approximate results from water not high in free carbonic acid may be obtained from samples collected in bottles which are completely filled so as to leave no air space under the stopper.

#### HALF-BOUND CARBONIC ACID.

When a water is acid to phenolphthalein, the half-bound acid is equal to 44 per cent of the alkalinity when the latter is expressed in terms of calcium carbonate.

When a water is alkaline to phenolphthalein, titrate 100 c.c. of the sample with  $\frac{N}{50}$  sulphuric acid, using phenolphthalein as an indicator.

Half-bound carbonic acid, indicating bicarbonates, is present only when the alkalinity by phenolphthalein is less than one-half of that by lacmoid or erythrosine. (See page 62).

Then twice the number of c.c. of  $\frac{N}{50}$  sulphuric acid required when phenolphthalein is used, subtracted from the number of c.c. of  $\frac{N}{50}$  acid used in determining the alkalinity of 100 c.c. of the water with lacmoid or erythrosine as an indicator (see page 61), multiplied by 4.4, gives in parts per million the half-bound carbonic acid as  $\text{CO}_2$ .

#### BOUND CARBONIC ACID.

Compute this in parts per million of  $\text{CO}_2$  as 44 per cent of the alkalinity by lacmoid or erythrosine when the latter is expressed in terms of calcium carbonate. (See page 61).

## DETERMINATION OF DISSOLVED OXYGEN.

There are three methods in use for the determination of atmospheric oxygen dissolved in water, viz., those of Winkler,<sup>83</sup> Thresh<sup>84</sup> and Levy.<sup>85</sup> Each of these methods has its own particular field of usefulness. All are capable of giving sufficiently accurate results.

The Winkler method is in the most common use in this country, and possesses the advantage of requiring only simple and not readily breakable apparatus. It is therefore recommended as the standard method.

The method of Thresh is perhaps slightly more accurate than the Winkler method, but the apparatus is not so well adapted to field work. For certain purposes, however, as, for example, the determination of dissolved oxygen before and after incubation, it is more practical than the Winkler method, because the apparatus allows the taking of representative samples direct from bottles or other containers.

What is true of the Thresh method is also true to a great degree of the Levy method. With both of these methods the samples are taken in a special, stoppered, separatory funnel.

*Reagents.*—1. Manganous sulphate solution: Dissolve 48 grams of manganous sulphate in 100 c.c. of distilled water.

2. Solution of sodium hydrate and potassium iodide: Dissolve 360 grams of sodium hydrate and 100 grams of potassium iodide in one liter of distilled water.

3. Sulphuric acid. Specify gravity 1.4 (dilution 1:1).

4. Sodium thiosulphate solution. Dissolve 6.2 grams of chemically pure recrystallized sodium thiosulphate in one liter of distilled water. This gives an  $\frac{N}{40}$  solution each c.c. of which is equivalent to .0002 gram of oxygen or 0.1395 c.c. of oxygen at 0° C. and 760 mm. pressure. Inasmuch as this solution is not permanent it should be standardized occasionally against an  $\frac{N}{40}$  solution of potassium bichromate as described in almost any work on volumetric analysis. The keeping qualities of the thiosulphate solution are improved by adding to each liter 5 c.c. of chloroform and 1.5 grams of ammonium carbonate before making up to the prescribed volume.

5. Starch solution. Mix a small amount of clean starch with cold water until it becomes a thin paste, stir this into 150 to 200 times its weight of boiling water. Boil for a few minutes, then sterilize. It may be preserved by adding a few drops of chloroform.

*Collection of the Sample.*—The sample shall be collected with extreme care in order to avoid the entrainment of any oxygen from the atmosphere. The sample bottle shall be preferably a glass stoppered bottle which has a narrow neck and which holds at least 250 c.c. The exact capacity of the bottle shall be determined and for convenient reference this may be scratched upon the glass with a diamond.

If the sample is to be collected from a tap the water shall be made to enter the bottle through a glass or rubber tube which reaches to the bottom of the bottle, the water being allowed to overflow for several minutes, after which the glass stopper is carefully replaced so that no bubble of air is caught beneath it.

If the sample is to be collected from the surface of a pond or tank two bottles shall be used, the ordinary sample bottle and a second bottle of four times the capacity. Both bottles shall be provided with temporary stoppers of double perforation and in both cases a glass tube shall extend through one hole of the stopper to the bottom of the bottle and a short glass tube shall enter the other hole of the stopper but not project into the bottle. The short tube of the sample bottle shall be connected with the long tube of the larger bottle. In collecting the sample the sample bottle shall be immersed in the water and suction applied to the short tube of the large bottle and enough water drawn through the hole to fill the large bottle. In this way the water in the smaller bottle will be changed several times and a fair sample secured.

If the sample is to be taken at a depth below the surface both bottles may be connected, lowered to the desired depth, and if the smaller bottle is placed beneath the larger one the water will enter the small bottle and pass from that into the larger bottle, the air escaping from the short tube of the large bottle. As soon as the small bottle has been filled remove the temporary stopper

and insert the permanent glass stopper using care not to entrain any bubbles of air.

*Procedure.*—Remove the stopper from the bottle and add approximately two c.c. of the manganous sulphate solution and two c.c. of the sodium hydrate-potassium iodide solution delivering both of these solutions beneath the surface of the liquid by means of a pipette. Replace the stopper and mix the contents of the bottle by shaking. Allow the precipitate to settle. Remove the stopper, add about two c.c. of sulphuric acid and mix thoroughly. Up to this point the procedure shall be carried on in the field but after the sulphuric acid has been added and the stopper replaced there is no further change and the rest of the operation may be conducted at leisure. For accurate work there are a number of corrections necessary to be made, but in actual practice it is seldom necessary to take them into account as they are ordinarily much less than the errors of sampling. Rinse the contents of the bottle into a flask, titrate with  $\frac{N}{40}$  solution of sodium thiosulphate using a few c.c. of the starch solution toward the end of the titration. Do not add the starch until the color has become a faint yellow; titrate until the blue color disappears.

*Calculation of Results.*—The standard method of expressing results shall be by parts per million of oxygen by weight.

It is sometimes convenient to know the number of c.c. of the gas per liter at 0° C. temperature and 760 mm. pressure and also to know the percentage which the amount of gas present is of the maximum amount capable of being dissolved by distilled water at the same temperature and pressure. All three methods of calculation are therefore here given.

$$\begin{aligned} \text{Oxygen in parts per million} &= \frac{0.0002N \times 1,000,000}{V} = \frac{200N}{V} \\ \text{Oxygen in c.c. per liter} &= \frac{0.1395N \times 1000}{V} = \frac{139.5N}{V} \\ \text{Oxygen in per cent of saturation} &= \frac{200N \times 100}{V \times O} = \frac{20000N}{VO} \end{aligned}$$

Where N = number of c.c. of  $\frac{N}{40}$  thiosulphate solution.

V = capacity of the bottle in c.c. less the volume of the manganous sulphate and potassium iodide solution added (i. e., less four c.c.).

O = the amount of oxygen in parts per million in water saturated at the same temperature and pressure. See Table 13.



TABLE 13.

QUANTITIES OF DISSOLVED OXYGEN IN PARTS PER MILLION  
BY WEIGHT IN WATER SATURATED WITH AIR AT  
THE TEMPERATURE GIVEN.<sup>86</sup>

Temp. C.	Oxygen	Temp. C.	Oxygen
0 .....	14.70	16 .....	9.94
1 .....	14.28	17 .....	9.75
2 .....	13.88	18 .....	9.56
3 .....	13.50	19 .....	9.37
4 .....	13.14	20 .....	9.19
5 .....	12.80	21 .....	9.01
6 .....	12.47	22 .....	8.84
7 .....	12.16	23 .....	8.67
8 .....	11.86	24 .....	8.51
9 .....	11.58	25 .....	8.35
10 .....	11.31	26 .....	8.19
11 .....	11.05	27 .....	8.03
12 .....	10.80	28 .....	7.88
13 .....	10.57	29 .....	7.74
14 .....	10.35	30 .....	7.60
15 .....	10.14		

DETERMINATION OF FATS.<sup>87</sup>

Evaporate 500 c.c. of sewage or other liquid in a porcelain evaporating dish to a volume of about 50 c.c. By means of a rubber-tipped glass rod remove to the bottom of the dish the solid matter attached to the sides, and add normal sulphuric acid to neutralize the alkalinity (with lacmoid). Do not use an excess of acid. Then evaporate the contents of the dish to dryness.

Treat the dry residue with boiling ether, rubbing the bottom and sides of the dish to insure complete solution of fat. Three extractions with ether are required.

Filter the ether solution through a five cm. filter into a weighed flask having a wide mouth. Evaporate the ether slowly, and put the flask in a desiccator for four hours or more. The increase in weight of the flask gives the amount of fats, or, in more precise language, the ether soluble matter.

Avoid an excess of acid, as it gives too high results due to acid fat residues.

DETERMINATION OF PUTRESCIBILITY.<sup>88</sup>

This test, sometimes called the "incubator test," is of fairly recent English origin. It is now quite generally used in connection with sewage works analyses. Its purpose is to ascertain whether or not the quantity of organic matter in a sewage effluent

of an unstable or putrescible character is in excess of that which can be oxidized by the oxygen which it contains in the form of dissolved oxygen or oxygen available from nitrates, nitrites, and perhaps sulphates. While it is a very useful and important test in connection with sewage purification the object of which is the elimination of gross nuisances, and in studying the details of a highly polluted stream, it is a test which obviously has no direct bearing in the regular field of water analysis, or even in the purification of sewage the effluent of which without further treatment is intended to enter a stream used a short distance below for drinking purposes.

*Procedure.*—A round, glass-stoppered bottle of good quality having a capacity of at least four ounces is completely filled with the sample, and after being tightly stoppered is placed in an incubator at 37° C. As the sample is collected determinations are made of the dissolved oxygen, nitrogen as nitrites and nitrates, and the oxygen consumed by digestion in an acid solution with potassium permanganate at the room temperature for a period of three minutes. After the sample has been incubated 24 hours (or more), observations are carefully made as to the appearance of the sample, that is, whether it has turned black or not, and particular attention is given to the presence or absence of well-defined odors of putrefaction. Sometimes a qualitative test for the presence of sulphuretted hydrogen may be made with advantage, by suspending in the mouth of the bottle a strip of filter paper saturated with lead acetate. Some make this test<sup>89</sup> quantitatively.

Samples which after incubation are black in appearance, due to ferrous sulphide, and which possess foul odors, may be unquestionably regarded as putrescible without making any further tests.

Samples which at the end of the incubation period still contain an appreciable quantity of dissolved oxygen, or oxygen available from nitrates, and are free from sulphuretted hydrogen or other odors resulting from putrefaction, may be generally regarded with safety as non-putrescible.

Samples in which dissolved oxygen and nitrogen in the form of nitrates are absent, or nearly so, with more or less nitrogen in the form of nitrites, and in which the oxygen consumed when

determined for three minutes in the cold has increased on incubation, require more careful consideration before recording definitely the result of the putrescibility test. The best procedures by which any additional information can be obtained appears to vary under different local conditions as to character of sewage treated, the method of treatment, season of the year, etc., and it seems inadvisable now to specify in precise terms further procedures for use under all circumstances.

As the applicability of this test is studied in various laboratories, it is recommended that reports set forth distinctly the procedures by which conclusions have been arrived at with reference to putrescibility.

It is to be noted that this test is a very rigid one for practical conditions, inasmuch as sewage effluents almost invariably are diluted more or less by the oxygen-containing waters of the streams which they enter. In some laboratories the practice is adopted of making the tests as above outlined and also repeating the same process with samples diluted with equal or varying volumes of river water. Where facilities permit, the adoption of this test also on a diluted sample is to be recommended.

The results shall be recorded simply as positive or negative, stating clearly whether the sample was diluted, and if so, to what degree.

*Note.*—In regard to the incubation temperature of 37° C., as compared with 27° C. as used in England, the committee is aware of the fact that the effluents of coarse-grained sewage filters contain at times more dissolved oxygen than can be retained without pressure at 37° C. By using round bottles of high grade, with well-fitting glass stoppers, it does not appear that any serious amount of dissolved oxygen is lost by using this temperature. Cork stoppers and bottles having a poor quality of glass, especially square bottles, which break easily, are not permissible. In England the bottles prepared for incubation are sometimes "jointed," that is, provided with a mercury seal. As yet this procedure has received very little if any attention in this country.

The evidence now available indicates that the numbers and kinds of bacteria are sufficient in the effluents of coarse-grained filters to preclude any thought that the use of blood temperature for incubation interferes with the reliability of this test as compared with its application at lower temperatures.

In regard to the use of a 24-hour period of incubation, available evidence indicates that this is sufficient as a general proposition, although it is true

that now and then the result would be modified if the period of incubation were extended. Such modifications do not affect those samples which are either clearly putrescible or clearly non-putrescible, but refer to those samples which are very close to one side or the other of the dividing line between putrescibility and non-putrescibility. It is prudent to treat conservatively this intermediate class last mentioned until further data are available to supplement the procedures above given.

#### MICROSCOPICAL EXAMINATION.<sup>90</sup>

The chief object of the microscopical examination of water is the determination of the presence or absence of those organisms which produce objectionable tastes and odors. In certain cases the examination is also of value as an index of pollutions, or as a guide to the identity of a water, i. e., whether surface water or ground water.

The term "microscopic organisms" shall be considered as comprising the diatomaceae, chlorophyceae, cyanophyceae, fungi, protozoa, rotifera, crustacea and other organisms microscopic in size, but not including the bacteria. Fragments of organic matter, broken down organisms, zoöglæa, etc., shall be termed amorphous matter. Clay, silt, oxide of iron and, in general, mineral matter shall not be included under amorphous matter, and shall not be measured by microscopic examination.

*Special Apparatus.*—1. A cylindrical funnel, about two inches in diameter, which has a capacity of about 500 c.c. and which terminates at the bottom in a tube about one-half inch in diameter, provided at the bottom with a perforated rubber stopper and a disk of silk bolting cloth just large enough to cover the hole.

2. A counting-cell consisting of a glass slide with a rectangular brass rim cemented on it, covered with a thin cover-glass; length of the cell, 50 mm., width, 20 mm., depth, 1 mm., capacity, 1 c.c.

3. An ocular micrometer consisting of a ruled square of such a size that, with the lenses used, it will cover an area of one square millimeter on the stage of the microscope. For convenience, this millimeter square is often subdivided into smaller squares.

*Procedure.*—Filter 250 c.c. of the water (more or less as the character of the sample demands) through a one-half inch layer of quartz sand (washed and screened between 60 and 100 mesh



sieves) placed at the bottom of the cylindrical funnel and supported by the rubber stopper and disk of bolting cloth. After filtration transfer the sand and the matter collected on it to a test tube, add 5 or 10 c.c. of distilled or filtered water and shake thoroughly. Pour off the liquid into a second tube. Put one c.c. of this concentrated sample into the cell, cover with a cover-glass, and allow the preparation to settle. Examine with a microscope, using a one-half inch objective and the micrometer above mentioned; count the organisms found in 20 squares taken at random in different parts of the cell. Knowing the degree of concentration and the number of squares counted, calculate the total number of organisms and express the results in number of standard units per c.c. One standard unit shall be considered as equal to a superficial area of 400 square microns. (One micron equals .001 millimeter.)

*Note.*—Inasmuch as many microscopic organisms are so fragile that they are liable to disintegrate during the process of filtration as above described, the regular procedure should always be supplemented by an ocular inspection of the original sample, followed, if necessary, by direct examination of the organisms. For the same reason the concentrated sample should not be allowed to stand long before the examination is made. Those microscopic organisms which are lighter than water rise to the top of the cell while the heavier organisms sink to the bottom. Care should be taken, therefore, in making the examination, to observe the entire depth of the liquid in the cell. If the cell is allowed to stand in front of a window some of the organisms may tend to move towards or away from the light and thus effect an unequal distribution of the contents of the cell. After counting the requisite number of cells it is therefore advisable to scrutinize the margins of the cell to see that no important organisms have been omitted from the record.

*Expression of Results.*—The results shall be expressed in whole numbers per c.c. The general directions as to significant figures given under Turbidity apply also to the microscopical examination.

## BACTERIOLOGICAL EXAMINATION.

### QUANTITATIVE BACTERIOLOGICAL DETERMINATIONS.<sup>91</sup>

In the present state of bacteriology there is no method known by which the absolute number of living bacteria in a sample of water can be determined, and all quantitative determinations of

bacteria are necessarily of a relative character. This being the case, strict adherence to a standard procedure<sup>92</sup> is of especial importance.

Single isolated determinations of the number of bacteria in a surface water are of little value, but have more weight when accompanied by a full knowledge of the conditions under which the sample was collected, since rainfall, stream-flow, wind and many other factors materially influence the number of organisms present. A single examination may therefore readily lead to erroneous interpretation. Sometimes, however, it may afford some evidence as to the sanitary character of water, and scattered determinations are often useful in showing the relative character at different times of water obtained from any particular source. Quantitative bacterial determinations are of especial value as affording the best index of the efficiency of filtration. Here each separate test is of some importance.

Before the standard mode of procedure described below was recommended, the relative advantages of many methods were carefully considered from all points of view and submitted to practical comparative tests. The current practice of the leading water laboratories was also ascertained by means of a circular letter, the answers to which were set forth at length in the Second Report of Progress of this Committee, made in 1901.<sup>93</sup>

*Media.*—The standard medium for determining the number of bacteria in water shall be nutrient gelatin, as is the case in Germany<sup>94</sup> and in England.<sup>95</sup> For field work, and for sewage and polluted waters which cannot be plated promptly after collection, agar may be substituted. All variations from these two media shall be considered as special media. If any medium other than standard gelatin is used, this fact shall be stated in the report.

For general work the standard reaction<sup>97</sup> shall be + 1 per cent, but for long continued work upon water from the same source the optimum reaction shall be ascertained by experiment and thereafter adhered to. If the reaction used, however, is different from the standard, it shall be so stated in the report.

The media shall be prepared as specified on pp. 104–110.

The use of simpler media,<sup>98</sup> such as albumose and agar dissolved in distilled water,<sup>99</sup> is a step in the right direction, but the evidence as to comparable results in various laboratories is still uncertain.

*Procedure.*—Shake at least 25 times the bottle which contains the sample. Withdraw one c.c. of the sample with a sterilized pipette and deliver it into a sterilized Petri dish, 10 cm. in diameter. If there is reason to suspect that the number of bacteria is more than 200 per c.c., mix one c.c. of the sample with nine c.c. of sterilized tap or distilled water. Shake 25 times and measure one c.c. of the diluted sample into a Petri dish. If a higher dilution is required proceed in the same manner, e. g., one c.c. of the sample to 99 c.c. of sterilized water, or one c.c. of the once diluted sample to nine c.c. of sterilized water, and so on. In the case of an unknown water or a sewage it is advisable to use several different dilutions for the same sample. To the liquid in the Petri dish add 10 c.c. of standard gelatin at a temperature of about 30° C., or 10 c.c. of standard agar at a temperature of about 40° C. Mix the medium and water thoroughly by tipping the dish back and forth, and spread the contents equally over the bottom of the plate. Allow the gelatin to cool rapidly on a horizontal surface and transfer to the 20° C. incubator as soon as it is hard. Incubate the culture for 48 hours<sup>100</sup> at a temperature of 20° C.<sup>101</sup> in a dark, well-ventilated incubator where the atmosphere is practically saturated with moisture.<sup>102</sup> After this period of incubation place the Petri dish on a glass plate suitably ruled and count the colonies with the aid of a lens which magnifies at least five diameters. So far as practicable the number of colonies upon the plate shall not be allowed to exceed 200. The whole number of colonies upon the plate shall be counted, the practice of counting a fractional part being resorted to only in case of necessity.

When agar is used for plating it will be found advantageous to use Petri dishes with porous earthenware covers\* in order to avoid the spreading of colonies by the water of condensation.<sup>103</sup>

*Expression of Results.*—In order to avoid fictitious accuracy and yet express the numerical results by a method consistent with the precision of the work the rules given below<sup>100</sup> shall be followed:

\*Made by Hews Pottery Co., Cambridgeport, Mass.

Numbers of Bacteria per c.c.

From	1 to	50	Recorded as found	
"	51	100	"	to the nearest 5
"	101	250	"	" " " 10
"	251	500	"	" " " " 25
"	501	1,000	"	" " " " 50
"	1,001	10,000	"	" " " " 100
"	10,001	50,000	"	" " " " 500
"	50,001	100,000	"	" " " " 1,000
"	100,001	500,000	"	" " " " 10,000
"	500,001	1,000,000	"	" " " " 50,000
"	1,000,001	10,000,000	"	" " " " 100,000

*Note.*—The determination of the number of bacteria which develop at 20° C. under anaërobic conditions, the number which develop at 37° C.,<sup>104</sup> the number of red colonies<sup>105</sup> which develop on a lactose litmus agar plate, and the number which develop on media other than the standard are not advised as regular procedures for either water or sewage. *B. coli* determinations are more valuable; species determinations may also be useful. No uniform methods of procedure for the special determinations above listed are here given, as the value of their determination depends upon the individuality of local conditions for each problem.

TEST FOR *BACILLUS COLI*.<sup>106</sup>

During the past decade the test for *B. coli* has increased more and more in importance. This organism is identical with, or at least very closely allied to, bacteria which are found somewhat widely distributed in nature,<sup>107</sup> and is essentially an organism whose habitat is the intestines of man<sup>108</sup> and of warm-blooded animals.<sup>109</sup> For this reason its presence in water is to some extent indicative of pollution, although its abundance rather than its mere presence must be considered as the criterion. The test for *B. coli*, in order to be of definite value, therefore, must be not only qualitative but quantitative.

*Quantitative vs. Qualitative Results.*—While colon bacilli in water cannot be determined quantitatively with such precision as can the mineral constituents, yet with sufficient care results may be obtained which are very valuable comparatively, and which are approximately quantitative. Qualitative results, when viewed superficially, may seem easier to obtain than quantitative and quite as conclusive; but detailed evidence shows that in general the quantitative tests are by far the more fruitful branch of study.



The committee therefore urges that more attention be given to this line of investigation, even if fewer samples are tested.

*Diagnostic Characters.*—The test for the presence of *B. coli* in water shall not be considered complete unless the organism is isolated in pure culture, in accordance with procedures described on pp. 90–91 and is found to show the following characters:

1. Typical morphology—non-sporing bacillus, relatively small and often quite thick.

Debilitated forms of colon bacilli sometimes show polar staining, giving the appearance of diplococcoid forms.<sup>110</sup> Such cultures show general physiological debilitation, including loss of motility, of power to ferment carbohydrates, to produce indol, etc. These and other facts accentuate the desirability of employing for this test methods which provide for regeneration by adequate preliminary cultivation.<sup>111</sup> Warning is especially directed against recording a negative result for the test as a whole, when abnormal features in some particulars have been encountered, until reasonable efforts have been made to restore the normal characters of a culture. The diplococcoid form above mentioned is not to be confused with sewage streptococci,<sup>112</sup> a separate group of bacteria often characteristic of pollution.

2. Motility—when a young broth or gelatin culture is examined.

Sometimes even young cultures of the colon bacillus do not show motility; but in these cases the process of rejuvenation,<sup>111</sup> will usually restore the normal character.

3. Non-liquefaction of gelatin.

Occasionally it may happen that the gelatin stab cultures are not allowed time enough to effect liquefaction. One week at a temperature of about 20° C. is a minimum period; ten days is a much safer one; while some workers prefer a period of two weeks. Some forms of *B. cloacae* liquefy only after a still longer period.

4. Fermentation of dextrose broth, with the formation of about 50 per cent of gas, of which about one-third ( $\text{CO}_2$ ) is absorbed by a two per cent solution of sodium hydrate.

Colon bacilli may give quite wide variations<sup>113</sup> in the amount of gas produced by the fermentation of carbohydrates. The above stated quantities of total gas and of  $\text{CO}_2$  (gas absorbed by sodium or potassium hydrate) are general averages. Deviations from these averages can usually be avoided by rejuvenation. In the fermentation of dextrose by colon bacilli the amount of gas formed often varies from 30 to 70 per cent, with a corresponding variation in the amount of  $\text{CO}_2$ . The use of carbohydrates other than dextrose in the fermentation tube is occasionally,

but not generally, important here. Lactose, however, is coming more and more into use in testing the water sample, prior to obtaining the species in pure culture.

5. Coagulation of milk, with the production of acid, in 48 hours or more at 37° C., either spontaneously or upon boiling.

Cultures are usually made in milk to determine acid formation and coagulation; but recent studies<sup>114</sup> indicate that such a procedure is unnecessary when a pure culture has already been tested for acid production in litmus lactose agar. Reddening of this medium invariably indicates that in milk the acid formation and coagulation will follow. Casein is not digested by *B. coli*. However, the committee advises that the use of milk be continued as a confirmatory test until further evidence is available upon the points just stated.

6. Production of indol in peptone solution.

Somewhat variable results with this reaction<sup>115</sup> have cast doubt upon the advisability of its continuance as a diagnostic test. Such variations, however, can be largely overcome by improved methods, including preliminary cultivation.<sup>111</sup>

7. Reduction of nitrates.

The statement regarding the indol test applies also here, though in a less degree.

*Quantities of Water Tested.*—For ordinary waters, 0.1, 1.0 and 10.0 c.c. shall be used for the colon test. For sewage and highly polluted surface waters, smaller quantities shall be used; and for ground waters, filtered waters, etc., the quantities shall be larger, if necessary to obtain positive results. The quantities shall vary preferably in the tenfold manner indicated. Single tests with quantities which give ordinarily a positive result or ordinarily a negative result are in themselves of but little account for quantitative determinations. The range in quantities studied shall be sufficient to allow the quantities needed for both a positive and a negative result to be recorded for each sample. When this is done, the results of several tests allow an approximate estimate of the number of *B. coli* per c.c.

*Present Procedures for Treating Samples.*—There are two methods in use by which water may be so tested as to obtain approximately quantitative determinations for colon bacilli. These may be termed, for convenience, A and B.

A. The preparation of an agar plate with a known volume of water, using a medium which contains a sugar and is colored blue with litmus. The plate is incubated at 40° C. Under these conditions *B. coli* and some other species show their presence by red colonies, due to acid fermentation of the carbohydrate. It must be remarked that not all red colonies are to be regarded as *B. coli* without further tests.

B. The preliminary cultivation at 40° C. of a known volume of water in a fermentation tube containing a sugar broth. If gas appears, a portion of the liquid is plated on agar, practically as in procedure A above, for the determination of *B. coli*. This procedure has quantitative value only when numerous portions of the water sample are tested to ascertain the approximate volume above which the results are positive and below which they are negative.

Each of these methods has some disadvantages, due in part to difficulties of technique. Both allow the use of only a very limited quantity of water, 5 to 10 c.c. at most; and the plate method, in testing polluted waters, necessitates the use of quantities of water so small that the red colonies are sufficiently separated to permit individual ones to be fished. Even when the plates are inverted, spreading colonies not only overgrow and obscure the red, but also render uncertain the question of pure cultures, and necessitate the labor of obtaining the cultures in purity after the colony has been fished. The use of Petri dishes with porous tops, however, largely overcomes this difficulty. As the method is commonly practiced, it has also the drawback of not affording a sufficient number of red colonies to serve as reliable data. In the fermentation tube method the disadvantages arising from overgrowths<sup>117</sup> and from the antagonism of other species are also present,<sup>118</sup> thus making somewhat difficult the task of obtaining later plate cultures of *B. coli*.\*

\*For the sake of explicitness the committee desires to call attention to the following definitions regarding growths of mixed cultures: Overgrowth means the predominance of one species over another as regards numbers of bacteria, but without necessarily any benign or prejudicial influence being exerted upon other species present. This term is also used with reference to extensive surface-film formation. Antagonism refers to the prejudicial influence of the growth of one species over another, while symbiosis involves a favorable influence of one species upon another.

To obviate these disadvantages, the following attempts at improvement in technique have been made, the discussion of which is pertinent here.

1. Concentration. As it is not possible by the procedures above described to treat directly more than 10 c.c. of a sample, and as filtered water (and some others) rarely contains *B. coli* in this volume, attention has been directed to the concentration of bacteria from relatively large quantities of water into volumes within reach of usual methods. This has been accomplished more or less satisfactorily in different ways, as follows:

a. By filtering through a sterilized filter tube of unglazed porcelain, and then removing the sediment by a sterilized filter brush with a small volume of sterilized liquid.

b. By means of centrifugalization.

c. By cultivating in a flask a mixture containing the desired volume of water with a suitable volume of sugar broth, and then if gas bubbles are noted, by treating some of the sediment in the usual way. Occasionally the sugar broths are made of increased strength so as to make the resulting mixture normal.

While these means of concentration have been helpful, they are not to be recommended, on account of the disturbances, due to overgrowth, etc., which they cause in securing quantitative data.

Where such waters are frequently studied it is advised that the fermentation tubes used be of such size as to allow at least 10 c.c. to be tested at a time, and that as many portions be tested as found necessary. It is obviously less expensive for laboratories connected with large water plants to obtain special apparatus for this purpose than to increase several fold the labor in getting reliable data.

2. Inhibiting Agents.—In addition to the use of a high temperature (the recent English report<sup>95</sup> strongly recommends incubation of the fermentation tube anaërobically at 42° C.), numerous efforts have been made to modify the composition of media so as to inhibit the growth of some of the bacteria commonly present in water and sewage. Attention has been given not only to various degrees of reaction, but to the addition of small quan-



ties of germicides, particularly phenol.<sup>118</sup> Opinion differs materially as to the value of the latter, indicating its special and not general applicability, and suggesting further that there ought to be recorded the results of comparative studies to show what species of bacteria are inhibited by its use.

Sodium taurocholate<sup>119</sup> (bile salt) is thought well of abroad<sup>95</sup> for inhibiting the growth of the more common spore-forming organisms, but in this country experience with it has been limited and uncertain.<sup>120</sup>

*Special Media for Colon Tests.*—General directions are given on p. 104 *et seq.*, for the preparation of the media. It is desired here also to call attention to the care necessary to prevent inversion of the sugar in preparing carbohydrate media. Solutions of litmus and of sugars are prejudicially affected by high temperatures. For this reason the intermittent method of sterilization should be used, and not the continuous or autoclave method. It is still safer to sterilize the azolitmin (and perhaps the sugars) separately at a low temperature, and to add them by means of a sterilized pipette after the media have been sterilized.

From present evidence it appears advisable when working under some conditions to exchange the sugars as commonly used in agar plates<sup>121</sup> and fermentation tubes.<sup>122</sup> In the latter it is highly desirable to restrict gas formation by other organisms than *B. coli*, and to this end lactose is more suitable than glucose or dextrose. In the agar plates, however, there are good grounds for believing that the gas-formers should make themselves apparent to the fullest extent, thus requiring *B. coli* to be identified from among the gas producers rather than from among the acid producers, as is the custom at present.

Each lot of agar, with its litmus and carbohydrate, should be thoroughly tested with a culture of *B. coli* to make sure that the conditions for gas formation and acid formation are facilitated as far as practicable. Now that spreading colonies are so nearly eliminated by the porous covers, the practice of each laboratory should be reconsidered with regard to the above mentioned points.

Nutrose agar<sup>123</sup> is thought well of abroad<sup>95</sup> and also in this country,<sup>120</sup> although some modifications in its preparation have

been made. While on present evidence the committee hesitates in advising its general use, it commends the investigation of its merits under a wide range of conditions.

The use of neutral red<sup>124</sup> has been found practicable by some investigators as an aid in isolating the colon bacillus, but while with certain waters this may be so, wider experience has shown the general unreliability of the method; its use is therefore not advised.

*Recommended Procedures for Treating Samples.*—Having in mind the facts given above, the committee has added the following details to methods A and B for treating water samples.

Procedure A.—This method is most applicable for sewages and polluted waters in which *B. coli* is present in one c.c. or less. It involves the preparation of agar plates in the usual manner as to dilution etc. (cf. p. 83), and with especial attention to the use of carbohydrate and litmus as above directed. Petri dishes with porous covers shall be used.

Incubate the plates at 40° C.\* for 12 to 24 hours. If no red colonies or gas-producing colonies appear, then *B. coli* is considered absent in the volume of the sample tested.

If colonies resembling *B. coli* are noted, then obtain a pure culture of each if the total number of such on the plate does not exceed five or six. If the number is larger, fish at least five colonies, but take them from an aliquot portion of the plate. The information afforded by a series of tests of the sample in fermentation tubes is frequently of sufficient aid in deciding upon the number of colonies to fish to make the preparation of these controls worth while.

As an optional adjunct to the ordinary plate method, cultures in purity may be obtained after a little practice by the use of streak plates. Nutrient agar is poured into Petri dishes and, if those provided with earthenware covers are not available the plates are allowed to remain in an inverted position, uninoculated, for 24 hours or so in order to obtain a relatively dry surface.

\* The committee advises that where practicable an incubator at 40° C. be provided for *B. coli* work; where a special incubator for *B. coli* work is not feasible the regular 37° C. incubator may be used, but a record should be made in each case showing the actual temperature used.

Inoculation is performed by drawing the needle several times across the surface of the agar without recharging it with inoculable material.

If the colonies obtained in pure culture resemble *B. coli*, then make sub-cultures upon a slanted agar tube (to preserve the culture for future study and reference), in fermentation tubes, milk, gelatin tube, peptone solution (for indol), and nitrate broth.

After examining the sub-culture, record the final result in accordance with the standard diagnostic characters stated on pages 85-86.

Procedure B.—This method is most applicable for waters requiring one or more c.c. to be tested for a positive result. It is also useful as a control for Procedure A, as just stated.

Transfer a measured quantity of the sample to two (or preferably more) lactose or dextrose fermentation tubes in accordance with the general directions of the foregoing pages.

Incubate the tubes at 40° C. for 48 hours, noting the presence of gas, if any, twice a day.

If no gas forms, record the result as negative.

As soon as gas has formed (usually in about 16-24 hours) plate at once in lactose litmus agar a portion of the sediment at the bottom of the closed arm, and continue the test as given for Procedure A.

It is necessary to note that while a method satisfactory in one laboratory for a given set of conditions can be used elsewhere for similar conditions, it by no means follows that such a method will be universally applicable. In fact, it is known that different conditions call for different details of procedure. Uniformity in this case must consist not in a blind adoption of a given technique, but in an adjustment of the underlying principles of the method to fit the conditions at hand.

#### PRESUMPTIVE TESTS.<sup>126</sup>

Partial tests for *B. coli* by which several but not all of the foregoing characteristics of the organism are ascertained are considered useful under some circumstances, especially where the

time allowed for making the test is necessarily limited. As a general rule, however, where tests for *B. coli* are needed it is believed that it is unwise to use methods giving less definite results than those described above.

#### TESTS FOR SEWAGE STREPTOCOCCI.

Some English bacteriologists are inclined to regard streptococci as indicating recent and objectionable pollution. In some cases it may be of value to test the sample of water for streptococci, but the information afforded by the occurrence of these organisms seems to be of less value than in the case of *B. coli* and the committee believes that, for the present at least, the streptococcus test is of subordinate importance.



DETERMINATION OF SPECIES OF BACTERIA.\*<sup>127</sup>

The determination of all the species of bacteria present in a sample of water or sewage is seldom practicable and the results of such determinations as are usually made, are at present of little direct value. This phase of water analysis is likely, however, to develop in the future and may eventually come to have greater importance.

Lack of uniformity, and indefiniteness<sup>128</sup> in description rendered a great part of the early work on bacterial species utterly valueless. It was this fact, indeed, that gave rise to the present movement for standard methods. The report of the Bacteriological Committee of 1897 has been a potent factor in this country in raising the standard of determinative bacteriology. The advantage of standard methods was practically illustrated by the co-operative work recently carried on by this committee and described in the appendix to this report.

In certain cases the determination of species may play directly a useful part in the analyses of water. It sometimes happens that the bacteria present in a filtered water are different in character<sup>129</sup> from those found in the raw water and unless the facts are known erroneous inferences may be drawn as to the efficiency of the filters. The determination of particular species is sometimes of importance in proving the identity of water from some particular source. At times also the presence of certain species in water may be indicative of pollution.

In the present state of our knowledge it is believed that information concerning the presence of streptococci, of *B. aërogenes capsulatus* (*B. enteritidis sporogenes*), of liquefying bacteria, etc., is not of sufficient value to warrant carrying out regular tests for these organisms. The determination of the presence of *B. coli*, however, is of such significance from the sanitary standpoint that a separate section of the report is devoted to that subject.

The occurrence of the typhoid bacillus and other pathogenic intestinal organisms is of course a matter of supreme importance, and, although it is usually impracticable to determine their

\* Revision of the 1897 Report.<sup>4</sup>

presence in water or sewage, except when they are very abundant, the question of selective methods is a vital one.

If the identification of various species of bacteria could be more easily accomplished and rendered quantitative within reasonable limits the results obtained by studying some of the more common and harmless bacteria would doubtless prove highly profitable. With this object in view the prescribed determinations which follow have been formulated so as to simplify these methods as much as possible and at the same time have them consistent with the accumulation of the data necessary for accurate identification.

The methods given are those especially applicable to the bacteria ordinarily found in water and sewage. They may be applied also to those bacteria that are involved in industrial processes. As applied to pathogenic bacteria, however, and to those species associated with the human body, they may prove somewhat deficient, but it is believed that even in the field of medical bacteriology the methods described will serve a useful purpose by systematizing results, and co-ordinating the work done by bacteriologists working in different fields.

Some of the biological tests described in the 1897 report of the Bacteriological Committee are here omitted. This is because they have not been found to be generally practicable, that is, the results are either not commensurate with the labor involved, or they are so variable under certain conditions as to have no diagnostic value.

It is becoming evident that in the future more detailed attention must be given to morphology and that the biochemical reactions must be made more precise. The hair-splitting methods of differentiation on the basis of arbitrarily selected cultural characteristics which were so common in the earlier days of the science must give way to more rational and broader ideas respecting the distinguishing marks of certain groups of bacteria, and it must also be recognized that these minute organisms are profoundly influenced by environmental conditions.

In the 1897 report the tests of bacteria were divided into two

groups,—necessary tests and optional tests. Practical experience has shown that most of the tests characterized as optional are of comparatively little importance in the study of water bacteria and that their use is extremely limited. The same may be said with respect to a few of the criteria formerly considered necessary, as, for example, the cultural characteristics on potato and blood serum. Of the necessary tests described in the former report only those have been retained which are considered to be of greatest importance. No doubt in particular cases some of the optional tests may yield data highly desirable and very instructive. These tests are described in the standard text books on bacteriology, and need not be detailed here.

In studying pathogenic bacteria found in water it is necessary to secure data in regard to the pathogenesis of the organism and to make studies regarding agglutination, etc. These are matters more particularly allied to medical bacteriology and while the need of their occasional use is recognized it is believed that they do not properly come within the sphere of this report.

The tests here recommended for differentiating species of bacteria may be divided for convenience into two groups—primary tests and supplementary tests. The primary tests are those which are of chief importance and which are necessary for differentiation in the majority of cases. They include those tests in which a definite positive or negative result may be obtained, thus enabling them to serve as a basis of classification. The supplementary data are ordinarily, but not always, of less importance than the primary tests. Sometimes they are highly desirable and nearly always instructive. It is recognized that this difference is an arbitrary and a provisional one, destined in time to give way when a more comprehensive system of taxonomy shall be developed; but it is believed that it fairly represents the present development of differential bacteriology and that it will serve for the immediate future.

The following data are required for the determination of bacterial species, and may be considered as representing the necessary minimum. Those which are classed as primary tests are indicated under Expression of Results (see p. 118).

- I. Source and habitat.
- II. Morphological characters.
  - Form.
  - Manner of grouping.
  - Dimensions.
  - Staining reactions:
    - a. with watery dyes; b. by Gram's method.
  - Presence or absence of flagella (motility).
  - Presence of spores and their character.
  - Fission.
  - Capsules.
  - Involution and degeneration forms.
- III. Cultural characteristics, mode of growth in and upon:
  - Nutrient broth.
  - Gelatin plates.
  - Gelatin tubes.
  - Agar plates.
  - Agar tubes.
- IV. Biochemical reactions.
  - Action upon milk (reaction and digestion of casein).
  - Action upon carbohydrates (fermentation, gas formation, production of acidity, etc).
  - Action upon nitrates.
  - Production of indol.
  - Inhibition of growth by acidity and alkalinity of media.
  - Relation to free oxygen (aërobic and anaërobic growth).
  - Temperature relations (activity of growth at 20° C. and at 37° C. and thermal death point).
  - Pigment formation.
  - Liquefaction of gelatin.

#### SOURCE AND HABITAT.

No uniform method or statement of facts regarding sources and habitat is possible. The data, however, shall be given with as much fullness as the case demands in order to obtain information for estimating the possible influence of environment.

#### MORPHOLOGICAL CHARACTERS.<sup>139</sup>

The morphological characters shall be determined and described from growths obtained upon at least one solid medium (nutrient agar), and in at least one liquid medium (nutrient broth). Growths at 37° C. shall be in general not older than 24 to 48 hours, and growths at 20° C. not older than 48 to 72 hours, yet it is important that the cultures be fully developed, as those which are too young may present immature forms due to rapid multiplication, while those which are old may contain altered or



degenerated forms. The growth from both solid and liquid media shall be examined both in stained preparations and in hanging blocks or hanging drops. Morphological descriptions shall be always accompanied by definite statements regarding the medium used and the temperature and age of the culture. It is important to examine the cultures both stained and unstained.

*Form and Grouping.*

Determine the form and grouping from growths on solid and in liquid media as stated in the foregoing paragraph.

*Procedure for Hanging Block.*<sup>131</sup>

Pour melted nutrient agar into a Petri dish to the depth of about one-eighth to one-quarter inch. Cool this agar and cut from it a block about one-quarter inch to one-third inch square and of the thickness of the agar layer in the dish. This block has a smooth upper and under surface. Place it, under surface down, on a slide and protect it from dust. Prepare in sterile water an emulsion of the organism to be examined, if it has been grown on a solid medium, or use a broth culture; spread the emulsion or broth upon the upper surface of the block as if making an ordinary coverslip preparation. Place the slide and block in a 37° C. incubator for 5 or 10 minutes to dry slightly. Then lay a clean sterile coverslip on the inoculated surface of the block in close contact with it, as far as possible avoiding air-bubbles. Remove the slide from the lower surface of the block and invert the coverslip so that the agar block is uppermost. With a platinum loop run a drop or two of melted agar along each side of the agar block, to fill the angles between the sides of the block and the coverslip. This seal hardens at once, preventing slipping of the block. Place the preparation in the incubator again for 5 or 10 minutes to dry the agar seal. Invert this preparation over a moist chamber and seal the coverslip in place with white wax or paraffin. Vaseline softens too readily at 37° C. allowing shifting of the coverslip. The preparation may then be examined as desired with a  $\frac{1}{12}$  inch homogeneous immersion objective.

*Procedure for Hanging Drop.*

Place a drop of fluid medium (or 0.85 per cent NaCl sol.) on a sterile cover-glass and inoculate the edge with a minute portion of the culture. Invert this preparation over a hollow slide, seal with vaseline and examine with a  $\frac{1}{12}$  inch homogeneous immersion objective.

Impression preparations of surface colonies are sometimes valuable. Determine the form also from stained preparations.

Until the labors of Migula, Fischer and others, who are seeking to place the classification of bacteria on a more scientific and substantial basis, shall have borne fruit, the old classification will be adhered to, namely:

*Coccus, or Micrococcus*.—(Forms which are spherical, or nearly so).

Single coccus, grouped irregularly.

Diplococcus, forming pairs.

Streptococcus, forming chains, often showing paired cocci.

Tetracoccus, forming fours by division through two planes.

Sarcina, forming packets of eight, or more, members, by division through three planes.

*Bacillus*.—(Cylindrical forms having one dimension decidedly greater than another, more or less straight and never forming spirals.)

Single bacillus.

Diplo- and strepto-bacillus, forming pairs and longer chains, the bacilli being attached end to end.

Filaments, or thread-like growths, in which divisions into bacilli of the normal length are not apparent, or occur irregularly and transversely to the long axis of the growth.

*Spirillum*.—Curved or twisted forms, constituting complete spirals or portions of spirals.

*Dimensions.*

Determine the dimensions of the bacterial cell both in stained preparations from agar cultures (made preferably with aqueous gentian violet), and in unstained hanging block or hanging drop preparations.

The most accurate way of determining the size of bacteria is by photography, but where this method cannot be used the measurements may be made by an ocular micrometer. To insure accuracy and uniformity measure the individuals just after fission has been observed and before a new fission takes place. Determine

the width, the average length and the extreme length. State the dimensions in microns and decimals of a micron (1 micron equals .001 mm.).

### *Staining.*

The primary object of staining bacteria is to make them more easily observed under the microscope with reference to their general morphology, but it has been found that different species react differently to similar stains, so that to some extent staining has a diagnostic value. The general process of staining is as follows:

*Reagents.*—1. An aqueous solution of fuchsin made by adding 5 c.c. of a saturated alcoholic solution of basic fuchsin to 95 c.c. of distilled water.

2. An aqueous solution of methylene blue similarly prepared.
3. An aqueous solution of gentian violet similarly prepared.
4. Alkaline solution of methylene blue:

Saturated alcoholic solution of methylene blue	-	30 c.c.
Potassium hydrate, one per cent solution	- -	1 c.c.
Distilled water	- - - - -	100 c.c.

5. Carbol fuchsin:

Basic fuchsin	- - - - -	1 g.
Alcohol	- - - - -	10 c.c.
Carbolic acid	- - - - -	5 g.
Distilled water	- - - - -	100 c.c.

Or this may be prepared by adding to a five per cent aqueous solution of carbolic acid enough saturated alcoholic solution of fuchsin to produce a metallic luster on the surface of the fluid. Filter before using.

6. Aniline water gentian violet. To 98 c.c. of distilled water add 2 c.c. aniline oil, shaking until the fluid becomes clear. Then filter and to 75 c.c. add the aniline water 25 c.c. saturated alcoholic solution of gentian violet. This solution will keep for two to three months if not exposed to strong light.

7. Iodine solution (Gram's):

Iodine	- - - - -	1 g.
Potassium iodide	- - - - -	2 g.
Distilled water	- - - - -	300 c.c.

*Procedure.*—Clean a cover-glass,\* removing the organic matter by immersing in a solution of potassium bichromate in dilute sulphuric acid, afterwards washing thoroughly. On the cover-glass make a very dilute emulsion of the culture in clean sterile tap water. Spread this as a thin film over the cover-glass and dry rapidly in the air. For ordinary purposes of observation fix the film by rapidly passing the cover-glass three times through a flame, but in the case of specimens to be used for measurement fix the film by heating in an automatically regulated air bath, taking care to keep the cover-glass from direct contact with the shelf of the oven. The difficulty of fixing preparations from liquid media may be overcome by allowing a few drops of 95 per cent alcohol to evaporate from the hot smear during the ordinary fixing processes, or by previously centrifugalizing the liquid culture. The latter method has the advantage of removing the debris of the medium from the smear. At least three stained preparations are required in all cases, namely: (1), made by using a watery dye; (2), by using aniline gentian violet; and (3), by using Gram's stain. Stain the preparation without heating; wash with clean water; mount in water or in balsam and examine under a magnifying power not less than that given by a  $\frac{1}{12}$  inch homogeneous immersion lens and a No. 3 Huygenian eye-piece.

Inasmuch as media of different refractive indices make differences in the picture obtained and inasmuch as no one medium is suitable for all cases, the kind of medium used shall be stated in all descriptions. The media most commonly used for mounting are water, balsam dissolved in xylol, or cedar oil, the former having a low index of refraction, the latter a high index. Balsam is recommended for general use.

In observing the stained preparations record the form and grouping, make the necessary measurements, note the internal structure of the organism as shown in both faintly and deeply stained specimens, and also the readiness with which the organism takes the stain. Make particular note of the presence of

\* For routine work the committee does not discourage the usual laboratory practice of making film preparations on slides instead of on coverslips.



vacuoles, capsules, spores, flagella, etc. These determinations in some cases require special modes of staining.

*Procedure of Staining by Gram's Method.*—Prepare and fix the preparation as above described and stain for one minute with aniline gentian violet. Wash the preparation and immerse for two minutes in Gram's iodine solution. Rinse in 95 per cent alcohol for five minutes; dry; mount in balsam and examine.

In determining the comparative staining capacities of different species, especially by Gram's method, it will sometimes be found convenient to mix the species under examination with two other species (e. g. *B. typhosus* and *B. diphtheriae*), one of which does and the other does not stain by the method used.

*Use of Acid Decolorizers.*—The application of acid decolorizers to stained specimens is sometimes of value in distinguishing bacterial species. Descriptions of these processes may be found in standard text books.

### *Flagella.*<sup>132</sup>

No single method is equally applicable to all cases but the following have been widely and successfully used:

#### *Method 1. (van Ermengem's).*

*Reagents.*—1. Solution A (fixative bath)—

Osmic acid, 2 per cent aqueous solution	-	-	-	-	1 part
Tannin, 10 to 25 per cent aqueous solution	-	-	-	-	2 parts

2. Solution B (sensitizing bath)—

Nitrate of silver, one-half per cent aqueous solution.

3. Solution C (reducing and reinforcing bath)—

Gallic acid	-	-	-	-	-	-	-	5 grams
Tannin	-	-	-	-	-	-	-	3 grams
Potassium acetate (fused)	-	-	-	-	-	-	-	10 grams
Distilled water	-	-	-	-	-	-	-	350 grams

*Procedure.*—Place the films in solution A for one hour at room temperature, or in a watch glass over a water-bath at 100° C. for five minutes. Wash with distilled water, then with absolute alcohol for three or four minutes, and again in distilled water. Transfer films to solution B contained in a shallow vessel, and agitate them for five seconds; then without washing transfer them to solution C in a similar dish, for five seconds with continuous

agitation, repeating baths B and C in rotation until specimens turn black-brown. It is now advisable to mount them in water and examine with a high power dry lens to ascertain the degree of staining acquired by the flagella; if not dark enough repeat baths B and C for a few times more, wash in distilled water, dry, and mount in balsam. It is advisable to renew baths B and C as soon as any deposit in them is noticeable.

*Method 2. (Bunge's).*

*Reagents.*—1. Mordant.

Five per cent aqueous solution of ferric chloride	-	-	-	1 part
Saturated aqueous solution of tannic acid	-	-	-	3 parts

2. Carbol-fuchsin.

*Procedure.*—Prepare a fresh agar streak culture. Remove some of the culture, being careful to avoid taking the culture medium, and add this to sterile tap (not distilled) water. Allow this to stand and diffuse rather than distribute it by stirring, thus preventing breaking the flagella. Should a liquid culture be used, centrifugalize it before withdrawing a portion of it for testing. It may be necessary to centrifugalize and decant several times before clearing the medium of all debris. Place some of the preparation on a scrupulously clean cover-glass and fix the film, taking great care to avoid overheating, as this may ruin the preparation. Treat over steam of a water bath with the mordant for two minutes; wash; and stain two minutes over water bath with carbol-fuchsin; wash; dry; and mount in balsam.

Describe the number and character of the flagella, noting particularly their location: in some of the new systems of classification these matters have a high diagnostic value.

*Spores.*

No process for staining spores is the best in all cases, but the following generally gives satisfactory results:

*Procedure.*—Prepare a cover-glass preparation from a 48-hour old culture. Stain by the usual methods and note the presence of spores, describing their size, their relation to the mother-cell and their location in the same. Note also the presence of free spores in the culture and determine their size and shape, whether

spherical, oval, elliptical, oblong, etc. If endospores are present they may be stained as follows:

*Moeller's Method.*—Place the films in chloroform for two minutes; then in a five per cent aqueous solution of chromic acid for two minutes; wash in water; cover with carbol-fuchsin and heat in the steam of a water-bath for five minutes; wash excess of stain off in water; decolorize not too vigorously in one per cent sulphuric acid; wash in water; counterstain with alkaline methylene blue for 10 seconds at room temperature.

The manner of germination of spores is of taxonomic importance and shall be studied by direct observation by the hanging block method.

*Note.*—In examining bacteria for the presence of spores care must be taken not to mistake for spores the small bright refulgent areas which are often seen and which usually do not take up the dye. In some cases these appearances are caused by vacuoles, fat drops or crystals. The only method of identification of spores which is absolutely positive is that of observing germination.

An approximate method of determining the presence of spores is to test the resistance of the suspected bacteria to moist heat at a temperature of 80° C. The procedure for this is described under "Temperature Relations." According to this method the presence of spores is indicated by the ability of the culture to develop after being subjected to a temperature of 80° C. for 15 minutes.

#### *Fission.*<sup>133</sup>

The recent observation that bacterial fission follows at least two distinct types, the ordinary method of division and that known as "snapping," the latter being a characteristic of the diphtheroid group, makes it advisable to examine all new species by direct microscopic observation in order to observe individuals undergoing multiplication. The hanging block method is well adapted to this purpose.

#### *Capsules.*

As the production of capsules is often dependent upon the presence of albumin in the culture medium the organism to be tested for capsules should be grown in milk or in serum media.<sup>144</sup>

*Procedure.*—Prepare the film from the medium without the use of water; fix; apply glacial acetic acid; drain immediately without washing; treat with the stain and examine in dilute salt solution.

*Involution Forms.*

Involution changes can be most readily recognized in stained preparations. The condition of both young and old cultures should be noted. Degenerate appearances are more likely to be observed in the latter; but in some cases they are found even in vigorous cultures.

## CULTURAL CHARACTERS.

Certain biological characters of bacteria are determined by cultivating the organisms in or upon various culture media and, after a certain period of incubation, noting, first, the mass characteristics of the organisms in the culture and, second, the biochemical reactions which they produce. Experience has shown that it is difficult to obtain consistent results upon these points because the nature of the growth is influenced by many conditions, such as the original vitality of the bacteria themselves, the composition and concentration of the culture medium, the period and temperature of incubation, the amount of moisture in the atmosphere, etc. Within limits, however, it may be anticipated that the more nearly these conditions can be reduced to uniform practice the more reliable will be the comparisons made between different investigations.

PREPARATION OF CULTURE MEDIA.<sup>134</sup>

In view of the marked influence upon bacteriological reactions of variations in culture media caused by differences both in ingredients and in technique of preparation, it is necessary that uniform methods be used in order to obtain comparable data. In specifying the various ingredients used in culture media it is the intention of the committee that they shall be uniform in quality, but it is not the intention that the recommendations as to ingredients and technical manipulations shall stand in the way of true progress as to improvements. When, however, improved or modified methods are used, the variations from the standard methods shall be plainly set forth together with the reasons for the modifications.

*Ingredients.*

Distilled water shall be used in the preparation of standard culture media.



Infusions of fresh lean meat, and not meat extract, shall be used as the basis of various media.

Sodium chloride shall not be added to any culture medium herein specified.

Peptone shall be that of Witte (dry from meat).

Gelatin shall be the best French brand, so-called. It shall be as free as possible from acids and other impurities, and shall be of such a character that a 10 per cent solution prepared in the usual way shall not soften when kept at a temperature of 25° C.

Commercial agar in threads shall be of as high a grade as can be obtained. Agar may be purified by washing.

The various sugars such as dextrose, lactose and saccharose, shall be as nearly as possible the chemically pure compounds designated. Unusual effort to obtain such sugars is considered to be necessary.

Glycerin shall be double distilled.

In place of litmus azolitmin shall be used as a one per cent aqueous solution.

Of the various other ingredients used, nearly all of which are of a mineral nature, special effort shall be made to see that they are chemically pure products within the full meaning of this expression.

#### *Sterilization.*

Of the two available methods of sterilization, the intermittent method at a temperature of 100° C. is considered on the whole to be preferable. The higher temperatures of the autoclave facilitate chemical reactions and changes which in some cases are undesirable.

When the latter method is used media contained in ordinary receptacles shall be sterilized by exposure in an autoclave at a temperature of 120° C. (15 pounds pressure) for five minutes. Where media are sterilized in large bulk, the period of heating shall be extended to 12 minutes. It is preferable, however, to sterilize media in reasonably small containers (500 to 700 c.c.).

In intermittent sterilization media shall be placed on each of three successive days in streaming steam for 30 minutes after the steam fills the sterilizer.

*Reaction.*<sup>97</sup>

Phenolphthalein shall be the standard indicator used in obtaining the reaction of all media. Turmeric paper possesses similar properties, and its use is advised where phenolphthalein is not available.

Titration and adjustment of reactions shall be made as follows:

Put five c.c. of the medium to be tested into 45 c.c. distilled water. Boil briskly one minute. Add one c.c. of phenolphthalein solution (five g. of commercial salt in one liter of 50 per cent alcohol.) Titrate while hot (preferably while boiling) with  $\frac{N}{20}$  caustic soda. A faint, but distinct pink color marks the true end-point. This distinct pink color may be more precisely described as a combination of 25 per cent of red (wave length approximately 658) with 75 per cent of white as shown by the disks of the color top, described under Record of Tints and Shades of Apparent Color, page 22. In practice titration is continued until the pink color of alkaline phenolphthalein matches that of the fused disks.

All reactions shall be expressed with reference to the phenolphthalein neutral point and shall be stated in percentages of normal acid or alkaline solutions required to neutralize them. Alkaline media shall be recorded with the minus (—) sign before the percentage of normal acid needed for their neutralization, and acid media with the plus (+) sign before the percentage of normal alkaline solution necessary for their neutralization.

The standard reaction of culture media shall be +1.0 per cent. If it differs from 1 per cent by more than 0.2 per cent it should be readjusted.

Wherever reactions other than the standard above given are used it shall be clearly stated in all results of bacterial work, and the reasons therefor also stated.

*Storage of Media.*

It is recognized by the committee that it is desirable to prepare media in large quantities in order to guard against discrepancies in composition; but, all things considered, the complications resulting from the varying amounts of heating incident to withdrawing portions from time to time and tubing it, are believed

to more than offset this advantage. Consequently, when possible, media shall be put at once into tubes and placed in cold storage.

To guard against changes due to evaporation all media not used promptly shall be stored in a moist atmosphere, preferably in an ice-box, or else the flask shall be sealed by dipping the cotton plug in paraffin.

#### *Nutrient Broth.*

Nutrient broth shall be prepared as follows: Infuse 500 g. chopped lean meat 24 hours with 1000 c.c. distilled water in refrigerator. Restore loss by evaporation. Strain infusion through cotton flannel. Add one per cent peptone. Warm on water bath, stirring until the peptone is dissolved. Heat over boiling water (or steam) bath 30 minutes. Restore loss by evaporation. Titrate. Adjust reaction to +1 per cent by adding normal hydrochloric acid or normal sodium hydrate, as required. Boil two minutes over free flame, constantly stirring. Restore loss by evaporation. Filter through absorbent cotton and cotton flannel, passing the liquid through until clear. Titrate and record final reaction. Tube, using 10 c.c. in each tube. Sterilize.

#### *Sugar Broths.*

Sugar broths shall be prepared in the same general manner as the standard nutrient broth, with the addition of one per cent of dextrose, lactose, saccharose or other sugar; or the sugar may be added to the completed nutrient broth just before sterilizing. Except in the case of dextrose broth it is important that the muscle-sugar in the meat infusion be removed by inoculating with *B. coli*.<sup>138</sup>

The reaction of sugar broths shall be neutral to phenolphthalein.

Sterilization shall be done in streaming steam in the case of all sugar broths to prevent inversion of the sugar.

For the routine work of testing samples of water for *B. coli* especially large volumes of water are to be mixed with broths of such strength, as to make the resulting mixture one of normal strength. Liebig's Beef Extract may be substituted for beef infusion in the preparation of dextrose broth only: three grams of the beef extract for each liter of broth.

*Nutrient Gelatin and Agar.*

Nutrient gelatin<sup>135</sup> and agar<sup>136</sup> shall be prepared as follows:

- | Gelatin.  | Agar.   |
|---|---|
| 1.  | Boil 15 g. thread agar in 500 c.c. water for half an hour and make up weight to 500 g. or digest for 10 minutes in the autoclave at 110° C. Let this cool to about 60° C. |
| 2. Infuse 500 g. lean meat 24 hours with 1000 c.c. of distilled water in refrigerator.  | Infuse 500 g. lean meat 24 hours with 500 c.c. of distilled water in refrigerator.  |
| 3. Make up any loss by evaporation.   |   |
| 4. Strain infusion through cotton flannel.  |   |
| 5. Weigh filtered infusion.   |   |
| 6. Add one per cent Witte's peptone and 10 per cent gold label sheet gelatin.   | Add two per cent of Witte's peptone.  |
| 7. Warm on water bath, stirring till peptone and gelatin are dissolved and not allowing the temperature to rise above 60° C.  |   |
| 8.  | To 500 g. of the meat infusion add 500 c.c. of the three per cent agar, keeping the temperature below 60° C.  |
| 9. Heat over boiling water (or steam) bath for 30 minutes.  |   |
| 10. Restore loss by evaporation.  |   |
| 11. Titrate, after boiling one minute to expel carbonic acid.   |   |
| 12. Adjust reaction to +1.0 per cent by adding normal hydrochloric acid or sodium hydrate as required.  |   |
| 13. Boil two minutes over free flame, constantly stirring.  |   |
| 14. Make up loss by evaporation.  |   |
| 15. Filter through absorbent cotton and cotton flannel, passing the filtrate through the filter until clear.  |   |
| 16. Titrate and record the final reaction.  |   |
| 17. Tube, using 10 c.c. of medium in each tube.   |   |
| 18. Sterilize five minutes in the autoclave at 120°, or for 30 minutes in streaming steam on three successive days. Put the gelatin at once into ice-water till solidified. |   |
| 19. Store in the ice-chest in a moist atmosphere, to prevent evaporation.   |   |



*Lactose (or Dextrose) Litmus Agar.*

Lactose or dextrose litmus agar shall be prepared in the same manner as nutrient agar, with the addition of one per cent of lactose (or dextrose) to the medium just before sterilization. The reaction shall be made neutral to phenolphthalein (see p. 106).

If the medium is to be used in tubes the sterilized azolitmin solution shall not be added until just before the final sterilization.

If the medium is to be used in Petri dishes the sterilized azolitmin solution shall not be added to the medium until it is ready to be poured into the dishes.

*Milk.*<sup>137</sup>

The milk to be used as a culture medium shall be as fresh as possible, "Certified Milk" being ordinarily the best obtainable in city laboratories. It shall be placed in a refrigerator over night to allow the cream to rise and the suspended matter to settle. The skimmed milk shall be siphoned off into a flask for use. It will be found more convenient, however, to allow the milk to stand in a separating funnel. Should the milk be too acid the reaction shall be corrected to +1 per cent by the addition of normal sodium hydrate. It is then ready to be tubed and sterilized. Litmus milk shall be prepared as above, with the addition of sterile 1 per cent azolitmin. As it is impossible to make each lot of litmus milk with the same shade of color, it is recommended that a control tube be always exposed with the inoculated tubes for purposes of comparison.

*Nitrate Broth.*

Dissolve one gram peptone in one liter of tap water, and add two grams of nitrite-free potassium nitrate.

It is convenient to prepare a stock solution of potassium nitrate by dissolving four grams of solid nitrate in 100 c.c. of distilled water and use five c.c. of this solution in the above formula. Ten c.c. of the medium thus prepared shall be placed in a test tube and sterilized in the usual way.

*Broth for Indol Test.*

Standard broth may be used for the indol test if precautions are taken to remove the muscle sugar,<sup>138</sup> by inoculating the beef infusion with *B. coli* before making the broth.

Peptone solution,<sup>139</sup> (one per cent peptone in water), however, is preferred by some for use in the indol test, and is considered generally to be satisfactory. Sodium nitrite (0.01%) shall be added in all cases.<sup>145</sup>

#### *Apparatus.*

Few definite requirements need be made respecting apparatus. The quality of the glass shall be such as not to be easily acted upon by the reagents used, and all glassware shall be scrupulously clean when used. When necessary it shall be sterilized by dry heat for one hour at about 150° C. A slight browning of the cotton stoppers is a good index of proper exposure.

In some operations, as, for example, the determination of the thermal death point it is necessary to use test tubes of a definite size and thickness. For this purpose the standard size culture tube shall be 15 cm. long, 1.6 cm. in diameter, and of medium weight. Tubes to be filled with gelatin for quantitative work may be those described as 6"  $\times$   $\frac{5}{8}$ " "heavy."

The standard loop for making transfers shall be prepared as follows:

Bend the end of a piece of No. 27 platinum wire about 10 cm. long over a bit of No. 10 wire, and fasten the loop thus formed into a glass rod to serve as a handle. A loopful of culture shall be interpreted as meaning all the fluid that the loop can hold. That is, the fluid shall form a bi-convex body and shall not be simply a film covering the space in the loop.

The standard fermentation tube shall be a tube 1.5 cm. in diameter, bent at an acute angle, closed at one end and provided with a bulb at the other which is large enough to receive all the liquid contained in the closed portion. The length of the closed end of the tube shall be about 8 cm.

#### *Incubation.*

There shall be two standard temperatures of incubation for special work, namely, 20° C. and 37° C., the first corresponding to ordinary room temperature, the second to blood heat. The temperature of the incubators shall not be allowed to vary from these two standards by more than 1° C. in either direction.

The atmosphere of the incubator shall be kept moist.<sup>102</sup> preferably near the point of saturation. The incubator shall be ventilated so as to insure a reasonably good circulation of air in order to prevent the accumulation in the incubator of gases which might be prejudicial to the development of the bacteria.

No definite period of incubation can be prescribed which will be suitable for all the work of species determination, but in reporting results the period used shall always be stated and form a part of the report. General statements as to the necessary periods will be found in connection with the principal tests.

#### *Preliminary Cultivation.*<sup>111</sup>

It is impossible to control completely the original vitality of bacteria when ready for cultivation, because in most cases the conditions for their optimum growth are not known. Experience, however, has shown that, when bacteria are submitted to a period of preliminary cultivation or rejuvenation in nutrient broth and transfers of young cultures made from one tube to another at frequent intervals, the result is to put the bacteria into a condition where subsequent cultures give greater uniformity in their characteristics than where this procedure is not followed. The following shall be considered as the standard procedure for this preliminary cultivation, and all bacteria shall be so treated before proceeding to the detailed tests.

*Procedure.*—Make a transfer from an agar culture of the bacterium to be tested into a tube of nutrient broth, and incubate for 24 hours at 20° C. Transfer from this culture to a second tube of broth, and again incubate for 24 hours at 20° C. Transfer from this second culture to a third tube of broth and incubate again for 24 hours at 20° C. From this third broth culture make a gelatin plate and incubate for 48 hours at 20° C. (This is to prevent working with a possible mixed culture due to accidental contamination.) From one of the colonies on the gelatin plate transfer to a tube of slanted agar, incubate at 20° for 48 hours, and use this culture for making subsequent inoculations in the various media.

## MODE OF GROWTH.

The following data represent the standard requirements for describing the mass characteristics of bacterial cultures.

*Nutrient Broth.*

Condition of fluid, whether clear or turbid; character of turbidity; amount of sediment, etc.; surface pellicle, color, consistency, structure; reaction and odor.

*Gelatin or Agar Plates.*

*Surface colonies.*—Form of colony; size of colony; surface elevation; topography of surface; microscopic internal structure of colony; microscopic structure of margin of colony; color; determined both by transmitted and reflected light; luster; consistency.

*Deep colonies.*—Form; size; microscopic structure; consistency; color; change in surrounding medium.

*Gelatin or Agar Tubes.*

*Stab cultures.*—Growth along line of puncture; surface growth (same as for plate cultures); extent of liquefaction, if any (same as for plate cultures).

*Streak cultures.*—Form; size; surface elevation; topography of surface; color; consistency; change in medium.

It is recommended that the descriptions of the cultures be of the briefest possible character, and that, as far as possible, botanical phraseology<sup>127</sup> be used.

Beyond this general recommendation no definite system of descriptive terms is hereby prescribed.

No definite period or temperature of incubation is prescribed in connection with the above determinations, but that time and temperature shall be used for each particular organism which appears to give the most characteristic cultures; and a statement of the general environmental conditions during cultivation shall form an integral part of the report. In general the period of incubation at 20° shall be four days, except in the case of gelatin tubes prepared to determine liquefaction, when the period shall be 14 days; at 37° the period shall be 48 hours.



## BIOCHEMICAL REACTIONS.

The following are the standard requirements for the biochemical study of bacterial species:

*Milk.*

This test is designed to show the effect of enzymes and other bacterial products upon the medium.

*Procedure.*—Inoculate the milk and incubate at 37° C. for two days. Observe the time required to curdle, the character of the curd, i. e., whether an acid or a rennet curd, the character of the whey, the digestion\* of the casein, the production of gas, and the odor.

*Note.*—In case no curd is formed, boil the milk for a few seconds and note whether curdling follows.

The reaction in a general way may be observed by adding one c.c. of sterile azolitmin solution to the milk before inoculation; but it must be remembered that the azolitmin itself may have an effect on the growth of the bacteria, and the practice is open to this objection. The reaction can be most accurately determined by titration, using phenolphthalein as an indicator.

*Action upon Carbohydrates.*

The action of bacteria upon the carbohydrates, i. e., upon dextrose, lactose, saccharose, etc., is best studied in the fermentation tube.

*Procedure.*—Just before using, heat the fermentation tube, containing the sugar broths, in the sterilizer, to drive off any dissolved oxygen that may be present. If on removal from the sterilizer gas bubbles are observed at the top of the closed arm remove them by decantation. When ready for use the liquid shall entirely fill the closed arm and stand at a low level in the bulb. When cool inoculate the surface of the liquid in the bulb with the culture to be tested. Incubate at 40° C. for 48 hours (or for a longer period if the gas formation is very slow). From day to day, however, observe the amount of gas, preferably with the aid of a gasometer.<sup>140</sup> At the end of the incubation remove the tube from the incubator and allow the liquid to come to room temperature. Express the amount of gas in per cent of the total volume of the closed arm. Note the degree of turbidity of the

\* Shrinkage of the curd must not be mistaken for digestion.

liquid in the closed arm, determine the reaction of the liquid in the bulb by the use of litmus paper, or more accurately by titration. Fill the open bulb completely with a two per cent solution of caustic soda and cover the end of the tube with the thumb. Decant the gas into the bulb and shake in order to allow the caustic soda solution to absorb the carbonic acid. Decant the gas back into the closed arm and measure the amount of gas remaining. The difference between this and the total amount of gas represents the carbonic acid. Express the result in the per cent which the carbonic acid bears to the total gas.

*Note.*—In the case of those bacteria which do not grow at 37° C. the test shall be made at 20° C. and the culture allowed to grow for at least four days. The rate of gas formation shall be determined by daily measurements.

The production of gas is often erratic but it is more constant when the organism to be tested has first been submitted to a preliminary cultivation.

#### *Action upon Nitrates.*<sup>141</sup>

The object of this test is to determine whether the bacteria in question will reduce nitrites and if so whether the nitrogen will be changed to nitrite, ammonia or free nitrogen.

*Procedure.*—Inoculate a tube of nitrate broth and incubate for four days at 37° C. together with an uninoculated tube to serve as a blank for comparison. At the end of that period remove three c.c. of the culture to a clean test tube and add two c.c. of each of the naphthylamine solution and the sulphanilic acid solutions described under the determination of nitrates (see p. 40). The development of a red color indicates the presence of nitrites, the amount of nitrites being in proportion to the intensity of the color. This should be compared with any color that may be produced by treating the blank in a similar manner.

Remove one-half of the remaining portion of the culture to a second test tube and test for the presence of ammonia by adding a few drops of nessler's solution (see p. 35). The presence of ammonia is indicated by a yellow color or precipitate. When these tests are positive no further observations are required but when negative one of two conditions may be present—either the nitrates may have remained unchanged or they may have been reduced to free nitrogen. This can be ascertained only by deter-

mining the presence of nitrates in the remaining portion of the culture, which may be done by means of the phenolsulphonic method described in this report on p. 40. In stating the results the facts shall be recorded as to whether the nitrates were or were not reduced and, if they were reduced, whether the final product was nitrites, ammonia or free nitrogen.

*Note.*—The test for the reduction of nitrates is sometimes quite erratic. In case it is suspected that ammonia has been produced it must be remembered that this may have come from the organic matter of the medium and not from the nitrates. This may be ascertained by testing a broth culture for the presence of ammonia and comparing the result with that obtained with the nitrate broth. In order for the test to be of greatest value these results should be made quantitative.

*Production of Indol.*<sup>115</sup>

The production of indol is determined from a broth culture from which all traces of muscle sugar have been removed, or from a culture in peptone broth.

*Procedure.*—Inoculate the broth and incubate for four days at 37° C. At the end of that time add two drops of concentrated sulphuric acid and one c.c. of a 0.01 per cent solution of sodium nitrite and allow to stand for half an hour. The appearance of a pink color indicates the presence of indol. A blank determination should always be carried on for comparison.

*Inhibition of Growth<sup>97</sup> by Reaction of Media.*

The effect of the reaction of the medium upon the growth of the bacteria may be determined in two ways, either by growth in broth cultures of different reactions or by plate cultures of gelatin having different reactions. The latter method has the advantage of giving not only the reactions which limit growth but of showing also the optimum reaction. The various media used for this test shall be prepared in series with reactions varying by 0.5 per cent on either side of the neutral point and the series shall extend in either direction as far as may be necessary to determine the limits of growth. Should nutrient broth or gelatin not be favorable media for the development of the bacteria in question, that medium shall be used in which it finds its most favorable growth and a statement of this fact shall be included in the results.

*Relation to Oxygen.*

The determination of the obligative and facultative properties of bacteria in relation to oxygen shall be established under conditions of free access to the atmosphere on the one hand, and, on the other, by exclusion of the air by one of the recognized methods of anaërobic culture.<sup>146, 147, 148</sup>

*Temperature Relations.*<sup>142</sup>

The main points to be determined in studying the relations of the bacteria to temperature are :

1. The comparative activity of growth at 20° C. and 37° C.
2. The extreme limits within which development occurs.
3. The most favorable temperature for development.
4. The thermal death-point of the bacteria both in the vegetative and in the spore stage.

The collection of all these data is often impracticable but at least the relative growth at 20° C. and 37° C. and the resistance of the organism to a temperature of 80° C. shall be determined.

*Procedure.*—Use a 48-hour old broth culture grown when possible at 37° C., or preferably make a preliminary investigation to see if cultures grown on other media are more resistant. First heat a capacious water bath to the desired temperature and place several tubes each containing 10 c.c. of sterile nutrient broth in the water in immediate contact with the thermometer. After 15 minutes exposure to this temperature inoculate the broth with three loopfuls of the broth culture to be experimented with by simply removing the cotton plug but not removing the tube from the bath. Expose the now inoculated tubes for 15 minutes at the desired temperature and then transfer them at once to a vessel of cold water in order to cool them rapidly and prevent further action of the heat upon the bacteria. When cold, place them at a temperature favorable for development and keep them under observation for not less than seven days to ascertain if growth occurs. The most accurate results can be secured by removing the contents with a sterile pipette and making plate cultures from the heated cultures. The temperature required to destroy the species under consideration shall be determined



within 2° C.; thus, if samples are exposed to temperatures of 50°, 52°, 54°, 56°, 58°, and 60°, and it is found that development occurs after an exposure to 56° but not after exposure to 58° and 60° the thermal death point shall be given as 58°, although further study might show that it was slightly less than this.

*Note.*—Bacteria in a desiccated condition offer a greater resistance to high temperature than when moist, hence the thermal death point test must always be made when the bacteria are in a moist condition. In old cultures the power of resistance of many or all of the cells may be somewhat diminished. The presence or absence of spores also affects the result. This may be determined by staining or an approximate idea may be obtained by noting whether or not the culture resists a temperature of 80°. The composition and reaction of the medium in which its resistance is tested, the amount of the culture medium used for the test, and the character of the containing vessel also affect the result. The standard size of test tube recommended is described on page 110. In case any departures are made from the prescribed procedure, as, for example, by the use of Sternberg's bulbs, they shall be stated with result.

#### *Pigment Formation.*

The formation of pigment by bacteria is usually apparent in agar culture, but some organisms occur which produce pigment only on certain special media, e. g., *B. mesentericus ruber*, on potato. The color is frequently less pronounced at 37° than at 20° C., as in the case of *B. violaceus*. In determining the production of pigment, therefore, observation should be made on different media and at both 20° and 37°. The presence or absence of oxygen may also affect the result.

In addition to the use of descriptive color terms,<sup>111</sup> record of the tint and shade of the pigment should be made by the more exact method described on page 106.

The distinction between the pigment color of a culture and its superficial luster should be made, as well as notes upon the limitation or diffusion of the color in the culture medium.

#### SUPPLEMENTARY TESTS.

Many other morphological, cultural and biochemical tests are useful in special cases, but they are either so variable or, at present, so imperfectly developed that they have not been included among the standard tests.

In certain cases agglutination tests<sup>149</sup> are important.

The use of potato has not been included because of its failure to give results of diagnostic value. The use of blood serum has been omitted for the same reason, although it may be valuable with certain pathogenic forms.

At times other carbohydrates than those specified may be used with advantage.

The tests for pathogenesis<sup>150</sup> are not considered necessary in the case of ordinary water and sewage bacteria.

#### EXPRESSION OF RESULTS.

Having accumulated the data prescribed on the foregoing pages it remains to codify them for purposes of reference and comparison. In the past verbose descriptions of cultural and biochemical characteristics have produced great confusion in bacteriological taxonomy. In order to simplify the labor of comparing different species and their biological characters and in order to distinguish so far as possible between those characters which are of permanent differential value and those which are due to accidental variations, various forms of classification<sup>143</sup> have been suggested. Many of these have much to recommend them but in practical use most of them have been found to be too complicated. In order for any system to be of the greatest use it is necessary that the various items of the schedule be so arranged that a definite, positive or negative result shall be recorded. From the experiments made by the committee and from data accumulated from individual experiments the schedule given on p. 119 has been prepared. This tabular statement shall be considered as embodying the primary tests necessary for the classification of bacterial species. The more minute botanical descriptions of the cultures and the details of the biochemical reactions shall be considered as supplementary to it and shall be separately recorded.

In the tabular summary of primary tests a single cross (+) indicates a positive result; a double cross (#) an emphatically positive result; a zero (0) indicates a negative result; a question mark (?) indicates that the determination was uncertain or variable; a blank space indicates that no determination was made.

The subject of the classification of bacteria is being considered by another committee of the association, and the above forms of expression are recommended for use only until this committee shall have fully completed its labors. It is believed, however, that no radical changes are likely to be made in the near future.

A practical illustration of the use of the system of recording the results of species determination is shown in the appendix to this report, which gives an account of some of the coöperative work carried on by the committee along this line.

## SCHEDULE OF PRIMARY TESTS FOR IDENTIFICATION OF BACTERIAL SPECIES.

NUMBER (OR NAME) FOR IDENTIFICATION.

Morphology	{	Form - - - -	{	Bacillus - - - -	+
				Coccus - - - -	0
				Spirillum - - - -	0
		Size - - - -	-	Diameter greater than 1 $\mu$ .	+
		Arrangement - -	{	United in pairs - -	+
				United in filaments - -	0
		Movement - - -	-	Motile - - - -	+
Structure - - -	{	Flagella observed - -	+		
		Spores observed - - -	0		
Staining reactions -	{	Stains easily with watery dyes	+		
		Stains by Gram's Method -	0		
Cultural characters - -	{	Broth tube - - -	{	Turbidity - - - -	+
				Sediment - - - -	+
				Pellicle - - - -	#
				Fecal odor - - - -	0
		Gelatin plate - -	-	Peculiarities of colonies -	0
		Gelatin tube - - -	{	Surface growth - - -	+
				Needle growth - - -	+
				Liquefaction - - - -	+
		Agar tube - - -	{	Dull growth - - -	-
				Wrinkled growth - - -	-
				Chromogenesis - - -	-
				Fluorescence - - - -	-
		Milk - - - -	{	Coagulation - - - -	+
				Degree of reaction - - -	0
Casein liquefaction - - -	0				
Dextrose broth - -	-	Gas formation - - -	+		
Lactose broth - - -	-	Gas formation - - -	0		
Saccharose broth -	-	Gas formation - - -	0		
Nitrate broth - - -	-	Nitrite formation - - -	+		
Peptone broth - - -	-	Indol production - - -	?		
Biochemical reactions -	{	Relation to oxygen -	{	Aërobic - - - -	+
				Facultative anaërobic - -	+
				Anaërobic - - - -	0
		Relation to temperature	{	Growth at 20° C. - - -	+
				Growth at 37° C. - - -	+
		Thermal death-point below 80° C.		- - - -	+

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## APPENDIX.

## COMPARATIVE STUDIES OF SPECIES.

Before recommending changes in the methods of determining species as set forth in the Report of the Bacteriological Committee of 1897, the Committee on Standard Methods of Water Analysis decided to make some practical tests of these methods, by ascertaining what results would be obtained by different observers working on the same species. On December 7, 1900, cultures of two species of bacteria isolated from the Brooklyn Water Supply were sent to 31 bacteriologists with the request that they determine the species, using the methods which they were accustomed to follow in their ordinary routine work. The cultures were selected almost at random from a gelatin plate and were marked A and B. Transfers were made to broth, then to gelatin plates and finally to agar slants, the latter being used for shipment.

Seventeen bacteriologists worked out these cultures in detail. Their results were presented in various forms and the labor involved in comparing them was in itself an excellent object lesson, showing the great need of uniformity of expression.

About a year later two other cultures were isolated from the Brooklyn Water Supply and sent out as before. These bacteria were marked C and D. This time, however, the request was made that each bacteriologist follow closely the procedures laid down by the Bacteriological Committee of 1897. Eleven series of results were reported in May, 1902.

The results obtained by the different observers studying these four species are shown in tabular form on the accompanying sheets, according to the method finally recommended by the committee.

It will be seen from them that, for the most part, the results of the qualitative tests were in substantial agreement but that in a number of instances there were marked discrepancies in certain of the tests. For example, in the case of nitrate reduction by one of the species, six observers reported positive results and three negative results; while in the case of indol production, four observers reported positive results and nine negative results.



COMPARATIVE RESULTS OF BACTERIOLOGICAL STUDIES OF SPECIES "A," MADE BY SEVENTEEN DIFFERENT OBSERVERS.

REFERENCE NUMBER	MORPHOLOGY				CULTURAL CHARACTERISTICS										BIOCHEMICAL REACTIONS																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
	Form		Size	Arrangement	Motile	Movement		Structure		Staining Reactions	Broth Tube			Gelatin			Agar Tube				Milk						Dextrose Broth	Lactose Broth	Saccharose Broth	Nitrate Broth	Peptone Broth	Relation to Oxygen	Relation to Temperature																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
						Flagella Observed	Spores Observed	Stains Easily with Watery Dyes	Stains by Gram's Method		Turbidity (sediment)	Pellicle	Faecal Odor	Characteristic Colonies	Plate	Surface Growth	Needle Growth	Liquefaction	Dull Growth	Wrinkled Growth														Chromogenesis	Fluorescence																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
	Bacillus	Coccus	Spirillum	Diameter Greater than 1 $\mu$	United in Filaments	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Positive result; 0 = Negative result.

COMPARATIVE RESULTS OF BACTERIOLOGICAL STUDIES OF SPECIES "B," MADE BY SEVENTEEN DIFFERENT OBSERVERS.

REFERENCE NUMBER	MORPHOLOGY				CULTURAL CHARACTERISTICS										BIOCHEMICAL REACTIONS																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
	Form		Size	Arrangement	Motile	Structure		Staining Reactions	Broth Tube			Gelatin			Agar Tube				Milk			Dextrose Broth	Lactose Broth	Saccharose Broth	Nitrate Broth	Peptone Broth	Relation to Oxygen	Relation to Temperature																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
						Flagella Observed	Spores Observed		Stains Easily with Watery Dyes	Stains by Gram's Method	Turbidity	Pellicle	Fecal Odor	Characteristic Colonies															Plate	Tube	Liquefaction	Dull Growth	Wrinkled Growth	Chromogenesis	Fluorescence	Coagulation	Reaction Alkaline	Casein Liquefac- tion	Gas Formation	Gas Formation	Gas Formation	Gas Formation	Indol Production	Facultative Anaerobic	Growth at 37 C.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
1.....	Bacillus	Coccus	Spirillum	Diameter Greater than 1 $\mu$	United in Filaments	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

+ = Positive result; 0 = Negative result.

COMPARATIVE RESULTS OF BACTERIOLOGICAL STUDIES OF SPECIES "C," MADE BY ELEVEN DIFFERENT OBSERVERS.

REFERENCE NUMBER	MORPHOLOGY					CULTURAL CHARACTERISTICS										BIOCHEMICAL REACTIONS																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
	Form		Size	Arrangement	Movement	Structure		Staining Reactions	Broth Tube			Plate			Gelatin			Agar Tube				Milk						Dextrose Broth	Lactose Broth	Saccharose Broth	Nitrate Broth	Peptone Broth	Relation to Oxygen	Relation to Temperature																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
									Turbidity (sediment)	Pellicle	Fecal Odor	Characteristic Colonies	Surface Growth	Needle Growth	Liquefaction	Dull Growth	Wrinkled Growth	Chromogenesis	Fluorescence																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
1.....	Bacillus	Spirillum	Diameter Greater than 1 $\mu$	United in Filaments	Motile	Flagella Observed	Spores Observed	Stains Easily with Watery Dyes	Stains by Gram's Method	Turbidity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Positive result; 0 = Negative result.





The following table shows the percentage constancy of the results for the various tests :

PERCENTAGE CONSTANCY.

	(A)		(B)		(C)		(D)	
	+	0	+	0	+	0	+	0
<i>Morphology:</i>								
Bacillus .....	100	0	100	0	100	0	100	0
Diameter greater than 1 $\mu$ .....	0	100	0	100	0	100	80	20
United in chains .....	100	0	12	88	33	67	90	10
Motile .....	100	0	100	0	20	80	100	0
Flagella observed .....	100	0	67	33	33	67	100	0
Spores observed .....	0	100	0	100	0	100	100	0
Capsules observed .....	50	50	100	0	0	100	28	72
Vacuoles observed .....					33	67	25	75
Stains easily .....								
with watery dyes .....	60	40	67	33	100	0	100	0
Stains by Gram's method .....	0	100	0	100	14	86	90	10
<i>Cultural Features:</i>								
Broth .....								
Turbidity (sediment) .....	100	0	100	0	100	0	100	0
Pellicle .....	79	21	31	69	50	50	91	9
Fecal odor .....	37	63	33	67	60	40	27	73
Gelatin Plate .....								
Characteristic colonies .....	64	36	8	92	20	80	30	70
Gelatin Tube .....								
Surface growth .....	86	14	92	8	100	0	100	0
Needle growth .....	37	63	100	0	100	0	100	0
Liquefaction .....	100	0	13	87	0	100	100	0
Agar Tube .....								
Dull growth .....	7	93	6	94	0	100	100	0
Wrinkled growth .....	0	100	0	100	0	100	100	0
Chromogenesis .....	50	50	8	92	0	100	0	100
Fluorescence .....	9	91	7	93	20	80	0	100
Potato .....								
Visible .....	100	0	100	0	100	0	100	0
Luxuriant .....	86	14	83	17	100	0	16	84
<i>Biochemical Reactions:</i>								
Milk .....								
Coagulation .....	39	61	80	20	100	0	100	0
Reaction alkaline .....	33	67	0	100	0	100	15	85
Casein liquefaction .....	27	73	0	100	11	89	71	29
Blood Serum .....								
Liquefaction .....	30	70	12	88	0	100	80	20
Dextrose Broth .....								
Gas Production .....	27	73	88	12	100	0	0	100
Lactose Broth .....								
Gas production .....	12	88	25	75	100	0	0	100
Saccharose Broth .....								
Gas production .....	0	100	100	0	100	0	0	100
Nitrate Broth .....								
Nitrate reduction .....	67	33	90	10	100	0	100	0
Pepton Broth .....								
Indol production .....	31	69	15	85	33	67	29	71
Relation to oxygen .....								
Facultative anaerobic .....	100	0	100	0	100	0	70	30
Relation to temperature .....								
Growth at 20° C. ....	100	0	100	0	100	0	100	0
Growth at 37° C. ....	77	23	100	0	100	0	100	0
Pathogenesis .....	0	100	0	100	50	50	60	40

When the figures stand 100 in one column and 0 in another they indicate that all the results were alike; when they stand 50 and 50, they indicate that the results were equally divided. The table shows that nearly all of the tests are at times more or less erratic with certain species. Species A seemed to be more variable in its cultural features and biochemical reactions than the others. Of the tests which showed the greatest variations, the production of indol was perhaps the most marked. The average percentage of constancy

was 85 per cent, that is, on an average 85 per cent would give results in one direction and 15 per cent in the other. The average percentage constancy for the cultural and biochemical tests in the case of species A, was only 81 per cent, while in the case of species D it was 93.5 per cent.

These tabular results do not tell the whole story. Some of the details not included in the tables are given below. They emphasize the need of greater attention to details in the methods of species determination.

#### SPECIES "A."

*Source*.—Brooklyn Water Supply.

*Name*.—No one succeeded in identifying this form with any published description. One connected it with the *bacillus liquidus* group and one with the *cloacae* group: one mentions its resemblance to *Pseudomonas albus* (Zimmerman.) The name "*Pseudomonas nigra*" has been suggested as an appropriate one.

#### MORPHOLOGY.

*Shape*.—Short rods with rounded ends; almost oval in form.

*Grouping*.—On solid media it occurs singly or in pairs; in broth, short chains are numerous.

*Size*.—Diameter very variable; extreme lengths, 0.4 to 0.8  $\mu$ ; on agar, usual diameter is 0.6 to 0.75  $\mu$ ; in broth, 0.7 to 0.8  $\mu$ . Length is variable. Minimum, 0.7; maximum, 3.0; average, 1.25  $\mu$ .

*Capsules*.—Capsules were demonstrated in broth cultures by two observers; two other observers failed to observe them.

*Spores*.—No spores produced.

*Flagella*.—Four observers readily demonstrated flagella by Pitfield's method and Loeffler's method. Their descriptions substantially agree that there is a single polar flagellum, three to five times the length of the cell, thin and undulating.

*Motility*.—Active, darting movements.

*Staining phenomena*.—Stains well and uniformly with watery dyes; is decolorized by Gram's stain.

#### CULTURAL CHARACTERS.

*Broth tube*.—Broth turbid after one or two days at 20° C. After a period, variously stated from two to nine days, a thin, milky scum forms on the surface, adhering to the sides of the tube, but sinking when disturbed. There is a heavy, flocculent precipitate. Old cultures show a brownish discoloration, and have a putrefactive odor.

*Agar tube*.—After 48 hours at 20° C. a narrow, flat, moist, glistening, homogeneous growth of a dull white color appears. Later this becomes thicker and wider, with undulating margins and shining surface. The color becomes brownish and the medium discolored or "smoky." The medium finally becomes very dark and almost black. Some describe the color as reddish-black, others as greenish-black.

*Gelatin plate*.—The surface colonies at the end of 48 hours are generally described as round, five or eight mm. in diameter, crateriform, entire or having a minutely ciliate border; texture granular or grumose, greenish-white in

color. One observer mentions notable concentric rings of granular material, while another speaks of a central nucleus and a radially striate border. One speaks of a cottony sediment. Liquefaction progresses rather slowly, and the medium becomes discolored. The *submerged colonies* at the end of 48 hours are almost spherical, and about 0.5 mm. in diameter. They are coarsely granular. The borders have a loosely grumose structure, giving a jagged appearance like colonies of the amoeboid or proteus type.

*Gelatin tubes.*—Descriptions generally agree that after 24 hours there is a well defined growth along the line of puncture, wider at the top, with a crateriform depression due to liquefaction of the gelatin. Liquefaction progresses slowly in the upper portions of the tube, and a heavy precipitate settles in the liquefied gelatin. After four or five days the upper half of the tube is entirely liquefied, and the funnel below is filled with a dense yellowish-brown flocculent precipitate. Some observers state that liquefaction progresses slowly, and that the growth takes the form of a narrow funnel, with little surface growth. One observer noticed that the first generation brought about a much more rapid liquefaction of the gelatin than did succeeding generations.

*Potato.*—Descriptions generally agree that after 48 hours the growth is scant, thin, moist, and dull white. After four days it becomes luxuriant, thick, with lobed margin. The color at first deepens to a yellowish-brown, and ultimately becomes a very dark brown, while the potato becomes black.

#### BIOCHEMICAL REACTIONS.

*Temperature relations.*—The reports of the various observers showed some disagreement. Three stated that no growths occur at 37° C., while 10 stated that growth does occur. The following quotations are taken from various reports: "The growth is more rapid but less abundant at 37° than at 20° C." "Grows well at 20°, but poorly at 37° C." "Grows well at 37°, but better at 20° C." "Grows more rapidly at 37° than at 20° C."

*Milk.*—The reports disagreed considerably. Eight stated that no coagulation took place, while five stated that it did occur. Three stated that acid was produced, three that alkali was produced, and two that the reaction was not changed.

*Nitrate reduction.*—Reports disagreed. Three stated that the nitrates were not reduced and six stated that they were reduced. Most observers stated that the nitrates were reduced to ammonia, and that no reaction was given for nitrite, while one stated that nitrates were reduced first to nitrite and then to ammonia.

*Indol production.*—The reports disagreed. Four stated that indol was produced, eight that it was not produced.

*Action upon carbohydrates.*—Most of the observers stated that no gas was produced in dextrose, lactose, or saccharose broth. Four stated, however, that gas was produced in dextrose broth and one that it was produced in lactose broth. One stated that in glucose broth 23 per cent of total gas was produced in five days at 20° C. One stated that at 20° C. a small amount of gas was produced in dextrose and lactose broth in some cultures but not in others.

*Relation to oxygen.*—The organism is a facultative anaërobe.

*Chromogenesis and fluorescence.*—Five observers reported that there is no fluorescence or chromogenesis. Four reported that the organism is chromogenic, and one that it is fluorescent.

*Pathogenesis.*—This was tested on a guinea pig and on a house mouse, without fatal result.

#### SPECIES "B."

*Source.*—Brooklyn Water Supply.

*Name.*—Two observers connect the organism with the colon type, one with the *Bacillus superficialis* type.

#### MORPHOLOGY.

*Shape.*—Occurs generally as short rods with rounded ends; sometimes oval.

*Grouping.*—Occurs singly and in pairs; sometimes in chains.

*Size.*—Diameter generally stated as 0.7 to 0.8 microns. One observer gives it as 0.5 and one as 0.3. The average length is given as 1.25 to 1.75, but lengths given vary from 0.7 to 3.0 microns.

*Stain.*—Stains readily with aqueous dyes. Is decolorized by Gram's method.

*Capsules.*—Two observers mention a capsule, and one speaks of a zoöglea formation in broth. One states that a broad uniform capsule was demonstrated by Lowitz' method.

*Spores.*—None produced.

*Motility.*—Actively motile.

*Flagella.*—One observer states that the flagella are peritrichial and that the average number is four. One failed to observe flagella by Pitfield's method. One mentions a single flagellum that may arise from any part of the body. (Lowitz' method.)

#### CULTURAL CHARACTERS.

*Broth tube.*—The reports agreed that broth is rendered turbid, and there is a sediment, but practically no scum.

*Gelatin plate.*—The reports generally agreed that in 48 hours the surface colonies are flat, irregularly round, and one to two mm. in diameter. Color is pale yellowish-white, slightly brownish in center. Later the colonies have a raised center and a slightly raised, lobed margin. The surface is somewhat granular, dull, brownish-white and translucent. General character is colon-like. Sub-surface colonies are spherical, with no special characteristics.

*Gelatin tube.*—The reports generally agreed that there is a slight growth along the track of the needle, which, after a time, becomes dense and beaded. The surface is somewhat similar to that on the gelatin plate. One observer mentions the formation of gas bubbles. Another states that liquefaction began on the twelfth day, and at the end of four weeks was noticed the whole length of the stab. Another states that in three weeks the gelatin was slightly liquefied, and in four weeks entirely liquefied.

*Agar tube.*—The reports generally agreed that there is at first a thin, moist, flat growth, grayish and translucent. Later it spreads widely as a flat



growth, with undulating margins, grayish and translucent. One report mentions a greenish fluorescence by artificial light.

*Potato*.—The reports generally agreed that after 48 hours the growth is thin, whitish, and shining. Later it becomes thicker, yellowish-white, moist, glistening, and luxuriant.

#### BIOCHEMICAL REACTIONS.

*Milk*.—All but two reports agreed that milk is coagulated with the production of acid.

*Nitrate reduction*.—All but two reports stated that nitrate is reduced. One stated that the reduction was to nitrites, another that it was to ammonia, and a third that it was reduced to both.

*Indol*.—All but two observers stated that no indol was produced. One report stated that "in neutral bouillon in five days there was no reaction cold, but it was evident on warming; in fourteen days there was a slight reaction cold, which became strong in warming."

#### Effect on Carbohydrates.

*Dextrose*.—All reports but two stated that gas is produced. Amounts of total gas vary from 42% to 77% in three days' incubation at 20° C. At 37° C. the amount of gas is stated as 84% to 90%. The average is about 60%. Reports of percentage of CO<sub>2</sub> agree quite closely. The average is 60% CO<sub>2</sub>, but variations from 57% to 67% were noticed. There was some difference of opinion as to the production of acid. Three reports stated that the liquid in the open bulb was alkaline, two that it was acid.

*Lactose*.—Only two reports mentioned gas production in lactose broth. One of these states that there was 20% produced in three days at 20° C. The other states that 6% was produced.

*Saccharose*.—All agreed that gas is produced in saccharose broth. The amount of gas is variously stated from 50% to 100%; the average being about 70%. The per cent of CO<sub>2</sub> ranged from 50% to 66%, the average being about 60%. The same disagreement was recorded as to the end reaction of the medium, as was noted under dextrose.

The following is a summary of the results of gas production in the various sugar broths:

Sugar	Bacteriologists number	Incubation days	Temperature, Degrees Cent.	Per cent total gas	Per cent CO <sub>2</sub>	End reaction
Dextrose.....	1	?	20	72	60	Acid
Dextrose.....	5	10	20	60	67	Alkaline
Dextrose.....	5	3	37	83	60	"
Dextrose.....	6	10	20	47	67	.....
Dextrose.....	9	5	20	77	57	Alkaline
Dextrose.....	10	10	20	80	..	.....
Dextrose.....	11	3	20	42	..	.....
Lactose.....	1	?	20	10	0	Acid
Lactose.....	10	10	20	6	..	.....
Saccharose.....	1	?	20	98	60	Acid
Saccharose.....	6	10	20	62	50	.....
Saccharose.....	9	5	20	100	..	Alkaline

*Temperature Relations.*—All observers agreed that the organism grows well at 37° C. and 20° C.

*Relation to Oxygen.*—All observers agreed that the organism is a facultative anaërobe.

*Chromogenesis and Fluorescence.*—All but two observers gave negative replies. One mentioned a fluorescence on agar which is lost on old cultures. One mentioned a yellow growth on potato.

*Odor.*—Two mentioned a disagreeable odor. Five stated that there was no disagreeable odor.

*Pathogenesis.*—One test; negative.

#### SPECIES "C."

*Source.*—Brooklyn Water Supply.

*Name.*—One observer stated that the species belonged to the colon group but was not identical with the *B. coli*; another thought it to be *bacillus lactis aerogenes*.

#### MORPHOLOGY.

*Shape.*—A short, stout bacterium, with rounded ends, variable in shape and size even in the same culture. On solid media it becomes very short, almost a coccus.

*Grouping.*—Single, often in pairs (resembling diplococci), and rarely in chains. Upon these characteristics there was universal agreement. One observer reported that the coccoid forms were most numerous in old cultures.

*Size.*—The diameter, as determined from agar culture, was variously stated as follows: 1.0; 0.8; 0.9; 1.0; 0.87; 0.74; 0.7; 0.3 microns. The average was 0.85 microns. The length on agar was stated as varying from one to three, the average being approximately two. On gelatin the dimensions were reported as being somewhat less than the above, and in broth, somewhat greater.

*Structure.*—Spores, capsules, vacuoles, crystals, etc., were generally reported as absent. One mentioned a "clear streak surrounding the cells." Another stated the "vacuoles were apparent in watery stain from a 48-hour agar culture." One observed vacuoles.

*Flagella.*—One observer reported that flagella were stained by Loeffler's method. Other observers reported no flagella.

*Motility.*—There was universal agreement that the bacterium was non-motile in broth. One observer reported a "sluggish motility in emulsions made from agar cultures grown 24 hours at 37° C."

*Staining Phenomena.*—Stains readily with watery dyes. One reported that it could be stained by Gram's method, six reported the contrary.

#### CULTURAL CHARACTERS.

*Broth Tube.*—At 37° C. the medium became visibly cloudy in four hours and distinctly turbid in 10 hours. It remained turbid, not clearing in 14 days. The observers disagreed somewhat as to the formation of a pellicle.

*Gelatin Plate.*—There was universal agreement that the gelatin was not liquified. Sub-surface colonies were called small, sub-spherical, on lenticular,

light colored, sometimes slightly yellow, with smooth margins and fine granular texture: Surface colonies were described as nearly round, with an entire, undulating or lobed margin, with a generally flat, but contoured surface and homogeneous granular texture, color generally whitish, sometimes translucent, near the edges. The gelatin colonies resembled those of *B. coli*.

*Gelatin Tube*.—The reports were in substantial agreement that growth took place along the stab, spreading out slightly over the surface, the lower part of the puncture was beaded. Some observers reported gas bubbles, others did not. One stated that the growth had a doughy consistency.

*Agar Tube*.—There was substantial agreement that growth took place as a raised culture with smooth, glistening surface and an undulating margin. Color almost white.

*Potato*.—The results were not in entire agreement. It was generally stated that the growth was heavy and moist. Some described it as dull, others as glossy. Some gave the color as a light yellow, some as dirty white and some as a faint green. Some stated that the potato was discolored around the growth, others that it was not.

#### BIOCHEMICAL REACTIONS.

*Temperature Relations*.—It was generally agreed that growth took place more rapidly at 37° C. than at 20° C. The thermal death-point was variously stated as follows, 54° C., 56°, 60°, and 62°.

*Milk*.—The results were in substantial agreement that milk was coagulated in 12 to 15 hours at 37° C. and in four or five days at 20° C. Acid was produced. Some stated that the curd was slowly digested, others that a granular sediment was observed after three days at 37° C. Some stated that the curd was soft and was not digested.

*Nitrate Reduction*.—(See Table.)

*Indol Production*.—(See Table.)

*Action upon carbohydrates*.—The results were in substantial agreement that gas was produced in all three sugars. The following figures show the maximum, minimum and average percentages of total gas and the relation which the carbonic acid bears to the total gas.

	Dextrose	Lactose	Saccharose
Per cent of { Maximum.....	84	85	85
total gas { Minimum.....	54	45	60
{ Average.....	70	70	70
Per cent which { Maximum.....	67	67	67
the CO <sub>2</sub> was of { Minimum.....	29	28	10
the total gas. { Average.....	52	52	47

*Relation to Oxygen*.—There was a general agreement that the organism is a facultative anaërobe.

*Chromogenesis and Fluorescence*.—(See Table.)

*Pathogenesis*.—(See Table.)

## SPECIES "D."

*Source*.—Brooklyn Water Supply.

*Name*.—One observer reported that the organism was *B. subtilis*; one reported that it belonged to the mesentericus group.

## MORPHOLOGY.

*Shape*.—Long rods in liquid media and short, stout rods on solid media.

*Grouping*.—Occurs singly and in chains of three or four individuals.

*Size*.—Diameter varies from 0.7 to 1.1  $\mu$ ; the average being between 0.85 and 0.95. Length varies, ordinarily from 2.0 to 4.0  $\mu$  but extremes vary from 1.5 to 10.0  $\mu$ .

*Spores*.—Small oval spores produced, located generally near the middle of the cell. These spores were studied somewhat carefully by Dr. Hibbert W. Hill, using the hanging block method: He reported that the free spore is elliptical and refractive, frequently showing remnants of an apparent membrane at one or both ends. The free spore enlarges rapidly in both diameters, loses its hard appearance and becomes practically indistinguishable from a very short vegetative rod. It quickly elongates and it is then possible to see at one end of it a developing rod. A faint line across the rod indicates the open end of the spore-case. The hard outline of the spore-case remains constant in size but the rod itself increases rapidly in length and diameter. After a time, the spore case slips off more or less quickly and smoothly from the enlarging rod, lying thereafter beside or behind the end of the rod but at a little distance from it, probably due to the slight jerk given as the growing tension of the cell membrane of the rod compels it to slip off. Frequently, even before the spore-case is "shed" the new rod will have divided by fission and these two rods, now separate at their proximal ends, one bowing a little and slipping past the other on the side opposite the concavity of the bow. (This "slipping by" occurs in both Species C and Species D.) The rods tend ultimately to become parallel but fission sometimes proceeds so rapidly that chains result, the component rods of which sometimes buckle out of line and then pass each other as above described. The rods in D often contain very numerous granules, which may be precursors of spores.

*Capsules*.—No capsules were observed by staining, but in some cases the reports stated that the bacteria were surrounded by clear zones.

*Flagella*.—Flagella were demonstrated by two observers. In both cases they were reported as peritrichic.

*Motility*.—The organism moves with a more or less direct undulating or wobbling motion.

*Staining Phenomena*.—The organism stains well and uniformly with watery dyes and also by Gram's stain.

## CULTURAL CHARACTERS.

*Broth Tube*.—Broth at first turbid, afterwards clear. Heavy light colored precipitate. Pellicle is formed which breaks easily and sinks to the bottom.

*Agar Tube*.—The reports generally agreed that the growth on agar was luxuriant, spreading, slightly raised, dull, wrinkled, opaque, white and slightly opalescent.



*Gelatin Plate.*—The descriptions generally agreed that the gelatin is liquefied, that the surface colonies are round and that cottony or slightly brownish masses float around in the liquid medium. Margin, rather smooth.

*Gelatin Tube.*—The descriptions generally agreed that liquefaction takes place first near the surface and ultimately proceeds down the tube in a stratiform manner.

*Potato.*—The growth on potato is a wrinkled dull dirty white mass.

#### BIOCHEMICAL REACTIONS.

*Milk.*—The reports generally agree that milk was promptly coagulated at 37° C. Most observers stated that subsequently the casein becomes digested.

*Nitrate Reduction.*—(See Table.)

*Indol Production.*—(See Table.)

*Action upon carbohydrates.*—The reports agreed that no fermentation of the sugars takes place.

*Relation to Oxygen.*—The organism was generally reported as a facultative anaërobic form, although a few observers described it as aërobic.

*Chromogenesis and Fluorescence.*—(See Table.)

*Pathogenesis.*—(See Table.)

## CONCERNING TESTS FOR *B. COLI COMMUNIS* IN WATER.

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AND

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THE writers of this paper were members of a joint commission\* which, on behalf of the city and of the water company, reported last autumn at Indianapolis upon the character of the public water supply. The investigation embodied a comprehensive study of the physical features of the water works system, an inquiry into the prevalence of typhoid fever and all the leading factors relating thereto, and a bacteriological examination of the city water supply. Daily tests of numerous samples of city water were made for a period of more than 30 days.

In the course of these investigations there were encountered a number of features which it is believed are worth recording briefly, although they are not essentially different from those experienced in some other instances. The points mentioned refer particularly to tests for *B. coli*, and these will be described following a brief outline of the works from which the public supply was at that time derived.

The major portion of the public water supply was derived from a series of deep wells, driven in the rock some 300 feet or so. The well water was delivered by a Pohle airlift system into an open reservoir, the sides and bottom of which were not water tight. Connected to this reservoir, which was quite near White River, was a gallery about 1,000 feet long, built diagonally along the shore and below the ordinary water level in the river. When the water was lower in the gallery than it was in the river, some river water passed into the gallery and reservoir after partial filtration through the intervening layers of porous sand and gravel.

\*See Report of Oct. 26, 1904, on the Water Supply and Sanitary Conditions of Indianapolis, by Messrs. G. W. Fuller, C. E. Ferguson and B. J. T. Jeup.

When the water in the gallery and reservoir became higher than it was in the river, there was obviously a flow in the opposite direction. During these investigations a portion of the new sand filter plant was put in service, and filtered water from Fall Creek formed a small part of the public supply.

The well water contains considerable iron, which very slowly deposits, forming within the pipes a sediment which becomes stirred up at times of very high velocities within the piping, such as during fires, etc. This sediment caused some irregularities in the bacterial contents of the water. To perhaps an unusual degree the water within the distributing system contained bacteria which were in a somewhat degenerate form. In part this seemed due to species which formed the 5 or 10 bacteria per c.c. present in the well water, coming in part, no doubt, from the air used in lifting the water from the wells. In part they seemed to be associated with the bacteria in the porous soil adjoining the reservoir and gallery, through which the water moved in opposite directions from time to time. Bacterial growths also took place to some extent in the gallery.

The number of bacteria per c.c. in water from different sources was found to average, for a period of 30 days, as follows:

	Bacteria per c.c.
White River - - - - -	350
Filter Gallery - - - - -	375
Gallery Reservoir - - - - -	120
Driven Wells - - - - -	4
City Tap Water - - - - -	100

# I.

The first point to which it is desired to call attention is the unreliability of the so-called presumptive colon tests under the existing local conditions. *B. coli* was repeatedly found to be present in fermentation tubes in which the amount of gas was less than 20 per cent or even 10 per cent after a period of incubation of 48 hours at 37° C.

The procedures followed in this work were substantially those recommended by the Bacteriological Committee of the American Public Health Association. Glucose solutions, however, were

prepared with meat extract rather than with meat infusion. The reaction of the media during the early part of the work was neutral to phenolphthalein, but later the reaction was made  $+0.5$  per cent. There was not much difference, however, in the two reactions used, although some special tests indicated that for this hard water (about 300 parts per million total hardness) the neutral reaction was not quite so satisfactory as in many places where softer waters are used.

In all 410 samples of water were examined for the presence of colon bacilli, in most cases one c.c. and five c.c. of each sample being tested. Colon bacilli were detected in 43 samples; 30 of them were in one c.c. portions, and the remainder in five c.c. portions.

In all cases there was a pure culture made from the fermentation tube and subcultures made on gelatin tubes, agar slants, dextrose broth (fermentation tube), milk, pepton solution (indol) and nitrate solution. Data from all these tests as well as the morphology were obtained before deciding on the identity of a species.

In 18 out of the 43 samples containing *B. coli* the volume of gas in the original fermentation tube was less than 20 per cent at the end of 48 hours, and in 11 instances it was less than 10 per cent. These figures indicate a serious error into which the investigation might have fallen if the records had been based on presumptive tests.

## II.

During the latter half of the investigation transfers were made on the morning of the second day into fresh fermentation tubes in the instance of each sample which had shown any sign of gas after having been incubated for a period of 15 to 18 hours at  $37^{\circ}\text{C}$ .

The second set of fermentation tubes was incubated for 48 hours at  $37^{\circ}\text{C}$ . The comparison of results so obtained with those from the tubes in which the samples of water were originally placed showed a variety of differences. In some cases the percentage of gas increased from below to within the conventional limits for presumptive tests, that is, 25 to 70 per cent; in others the gas increased from within to above the limits stated;



in others there were reversals in each of the two last mentioned sets of conditions; and finally there were occasional changes from below 25 to above 70 per cent, and, vice versa. In repeated instances, of course, the percentage of gas remained approximately the same.

Observations from day to day of the irregularities above described deeply impressed the writers with the uncertainties associated with the use of mixed cultures in obtaining such biochemical reactions. The effects of overgrowth, antagonism, symbiosis and other features which have been written about from time to time for several years are given an opportunity to exercise their influence in a manner which the writers believe is prejudicial, under many cases, at least, to obtaining reliable data as to the distribution and prevalence of colon bacilli in waters.

If two or more gas-producing organisms are present in the sample, there are no reliable means of determining in advance which will have gained the upper hand of the other at any particular time, although, as is frequently the practice, it is advisable to remove for examination a portion of a tube which shows signs of gas in 15 to 18 hours, rather than to allow from 24 to 48 hours to elapse before transferring a portion for examination.

When consideration is given to the effect in a fermentation tube of bacterial species other than those which produce gas, of relative states of vitality, etc., some comprehension is obtained of the hopelessness of arriving regularly at reliable data by these means.

In making the above statements the writers are aware that the degenerate forms of *B. coli* present in many waters behave quite differently in some respects in the laboratory from colon forms taken directly from sewage or from the bodies of men or animals.

### III.

Quite frequently it has been the experience that positive tests are obtained for colon bacilli in one c.c. volumes when negative results are recorded for larger ones. In the light of the experiences above described, and of our growing knowledge regarding the effect of overgrowths, antagonism, etc., it appears to the

writers that it is necessary to recast present views as to colon tests, making more numerous examinations of smaller volumes rather than to attempt to get anything reliable from the examination of larger unit volumes. This refers, of course, essentially to the use of the fermentation tube for the preliminary treatment of the water sample. Where feasible, the direct plating of the sample on agar seems preferable.

#### IV.

During the progress of this work the writers were unaware of the marked step in advance resulting from the use of porous earthenware covers<sup>1</sup> for the Petri dishes. Spreading colonies to rather an unusual degree were encountered in the Indianapolis work, thus making it a laborious task to obtain in purity cultures of the leading species producing gas in a fermentation tube. Under these circumstances great care is required, otherwise the spreading colonies will mask the presence of *B. coli*.

It appears that with the large elimination of spreading cultures by the absorption of moisture with earthen covers, there is an important field of investigation opened for improvement in the technique of coli determination. It would seem that more and more attention should be given to the direct plating of samples on agar, rather than to the use of the preliminary fermentation tube with the complications which it presents as above outlined.

It especially suggests the use of technique by which there may be isolated species producing gas, thus confining the work of searching for *B. coli* on agar plates to those gas-producing forms rather than to those which produce acid. With most workers the litmus agar plates now and then show well the gas-producing colonies, but the present technique does not seem to keep this feature uniformly under control. The use of other sugars than lactose in agar, and the testing before use of each lot of agar with a stock culture of *B. coli* to insure favorable conditions and technique for gas production, are measures which seem to promise better quantitative results.

<sup>1</sup> HILL, *Jour. of Med. Research*, 1901, 8, p. 93.

## CHARACTERISTICS OF COLON BACILLI AND THE VALUE OF THE PRESUMPTIVE TEST.

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*(From the Research Laboratory.)*

IN November, 1903, an investigation was begun under the direction of Dr. William H. Park, in order to study anew what has already been the subject of considerable research work, namely, the colon-like and sewage streptococcus types of normal, and incidentally of diarrheal stools; how far the typical *B. coli* organism can be safely used as an index of pollution; and whether the presumptive test, as still applied in some important laboratories, can be relied upon as giving a fair basis for a reasonably correct judgment of waters used as sources of drinking water supply.

In the first experiments nine specimens of normal human stools from as many sources were examined. In each case a flask containing 100 c.c. of sterile tap water was inoculated with a large loopful of feces, and plates made with .1 and 1 c.c. of water from each flask directly and at intervals varying from two days to a week and extending over a period of two and a half to three months.

It will be seen from Table 1 that seven, or 77 per cent of the first series of plates made from one c.c. of the diluted feces in the nine inoculated flasks show evidence of *B. coli*, and of the six plated at the end of six weeks, five still contain abundant colon bacilli. Of those plated at the end of 10 weeks, more than 50 per cent show *B. coli* to be still abundant.

From two of the nine original flasks no colon-like colonies, in fact no gas producers whatever, were obtained in the quantity used at the first plating; one gave them subsequently, while the other gave none, at any time during the 10 weeks that the flasks were under observation. It is interesting to note that organisms of the sewage streptococcus type were found to be present in

TABLE 1.

PERCENTAGE OF COLONIES OF COLON-LIKE AND SEWAGE STREPTOCOCCUS TYPES DEVELOPING IN NUTRIENT AGAR AT 37° C. FROM SUCCESSIVE PLATINGS FROM FLASKS INOCULATED WITH NORMAL STOOLS.

	FLASK 1			FLASK 2		
	Time	Per Cent of Colon-like Colonies	Per Cent of Sewage, Streptococcus Type	Time	Per Cent of Colon-like Colonies	Per Cent of Sewage, Streptococcus Type
First plating.....	0	84	4	0	6	6
Second plating.....	3 days	90	0	3 days	100	0
Third plating.....	8 "	78	0	8 "	96	0
Fourth plating.....	10 "	100	0	10 "	74	0
Fifth plating.....	16 "	100	0	16 "	26	0
Sixth plating.....	—	—	—	—	—	—
Seventh plating.....	—	—	—	—	—	—
	FLASK 3			FLASK 4		
	Time	Per Cent of Colon-like Colonies	Per Cent of Sewage, Streptococcus Type	Time	Per Cent of Colon-like Colonies	Per Cent of Sewage, Streptococcus Type
First plating.....	0	63	0	0	71	7
Second plating.....	2 days	0	0	2 days	63	18
Third plating.....	5 "	73	0	9 "	10	0
Fourth plating.....	15 "	89	0	16 "	0	0
Fifth plating.....	3 weeks	53	0	6 weeks	25	0
Sixth plating.....	7 "	60	0	10 "	5	0
Seventh plating.....	11 "	82	0	—	—	—
	FLASK 5			FLASK 6		
	Time	Per Cent of Colon-like Colonies	Per Cent of Sewage, Streptococcus Type	Time	Per Cent of Colon-like Colonies	Per Cent of Sewage, Streptococcus Type
First plating.....	0	100	0	0	0	12
Second plating.....	8 days	0	0	7 days	0	10
Third plating.....	15 "	0	0	14 "	0	92
Fourth plating.....	6 weeks	0	0	6 weeks	0	93
Fifth plating.....	10 "	0	0	10 "	0	75
Sixth plating.....	—	—	—	—	—	—
Seventh plating.....	—	—	—	—	—	—
	FLASK 7			FLASK 8		
	Time	Per Cent of Colon-like Colonies	Per Cent of Sewage, Streptococcus Type	Time	Per Cent of Colon-like Colonies	Per Cent of Sewage, Streptococcus Type
First plating.....	0 days	71	19	0	0	4
Second plating.....	7 "	50	0	5 days	26	4
Third plating.....	14 "	30	5	12 "	35	0
Fourth plating.....	6 weeks	93	0	6 weeks	80	20
Fifth plating.....	10 "	85	0	10 "	80	5
Sixth plating.....	—	—	—	—	—	—
Seventh plating.....	—	—	—	—	—	—
	FLASK 9			No streptococcus actually isolated in this case.		
	Time	Per Cent of Colon-like Colonies	Per Cent of Sewage, Streptococcus Type			
First plating.....	0	36	32			
Second plating.....	5 days	0	0			
Third plating.....	12 "	4	0			
Fourth plating.....	6 weeks	81	0			
Fifth plating.....	10 "	0	10			
Sixth plating.....	—	—	—			
Seventh plating.....	—	—	—			



every series of plates made from the flask containing no colon bacilli. Although this experiment is made with only nine samples of normal stools, the statement that *B. coli* will be abundantly present in waters subjected to multiple human pollution will be but a fair deduction from the table. It is also evident that in those cases where pollution from but one person occurred, and where *B. coli* was not found in any series of plates, that in the majority of cases organisms of the sewage streptococcus type would have given at least an indication of pollution.

*Agglutination Experiments.*—A group of 14 colon-like organisms, taken at random from the feces of the nine cases under discussion, and of course all of them unquestionably intestinal bacteria, were examined for agglutination reactions, and attempts were made to obtain with them an agglutinating serum for *B. coli* which should be diagnostic.

In carrying out this part of the investigation a large number of rabbits were inoculated by us with separate strains of coli, and several with mixed strains of coli cultures. The rabbits were bled and their serum tested for agglutinating reactions with the 14 cultures just mentioned. No original rabbit serum reacted strongly with any culture in a higher dilution than 1:50.

By successive inoculations of a mixture of two or three coli strains for each rabbit, it was found by testing the sera that specific agglutinins for just those strains which had been used could be raised.

Indeed, for the majority of the cultures, other than those injected, no appreciable amount of non-specific or group agglutinins developed. Only a few cultures of those not injected agglutinated slightly in the sera in 1:20 dilutions, and in most cases these cultures were derived from the same person as one of the cultures used in immunizing the animals. This result does not harmonize with the belief expressed by some that even though cultures of *B. coli*, isolated from different persons, reacted to different specific agglutinins, yet among the total specific agglutinins acting on each *B. coli* there would be one or more acting on all or at least on a large number. In our experience no more common agglutinins are found among many different

cultures of *B. coli* than are common to a strain of *B. coli* and cultures of the mannite fermenting para-dysentery bacilli.

The results of our work will be found in Table 2.

TABLE 2.  
AGGLUTINATION REACTIONS WITH GROUP OF 14 COLI STRAINS FROM NORMAL STOOLS.

RABBIT 1 (Inoculated with Coli Strains 1, 7 and 12)					RABBIT 2 (Inoculated with Coli Strains 3, 4 and 10)				
	1:100	1:200	1:500	1:1000		1:100	1:200	1:500	1:1000
1 .....	+1	+	±		3 .....		+1	±	1
7 .....	+1	+1	±		4 .....	++	+	±	1
12 .....	+	+	±	1	10 .....	++	++	+1	+
With eleven other Coli strains a negative or only slight reaction was obtained at 1:20. ++ signifies a complete reaction. + signifies a good reaction. 1 signifies a slight reaction.					With strains 1 and 2 a fair reaction was obtained at 1:50; with nine other Coli strains a negative or only slight reaction was obtained at 1:20.				
RABBIT 3 (Inoculated with Coli Strains 2, 8 and 13)					RABBIT 4 (Inoculated with Coli Strains 5, 6 and 14)				
	1:100	1:200	1:500	1:1000		1:100	1:200	1:500	1:1000
2 .....	++	++	++	±	5 .....	+1	+1		
8 .....	+1	+	+		6 .....	±	±	±	±
13 .....	+	±	±		14 .....	±	1		
With strains 1 and 7 a fair reaction was obtained at 1:50; with 12 a fair reaction at 1:20; with eight others a negative or only slight reaction was obtained at 1:20.					With strain 1 a fair reaction was obtained at 1:50. With eleven others a negative or only slight reaction was obtained at 1:20.				
RABBIT 5 (Inoculated with Strains 9 and 11)									
	1:100	1:200	1:500	1:1000					
9 .....	+	±							
11 .....	±	1							
With strain 10 a fair reaction was obtained at 1:50. With eleven other cultures only a negative or slight reaction was obtained at 1:20.									

Other rabbits were inoculated with single strains, but in no case were agglutinins developed which were specific for members of the coli group. The sera obtained were only specific for the single strain used, and possibly specific to a smaller extent to another organism from the same specimen of stools.

Horse sera obtained by the inoculation of dysentery cultures was used to test these fourteen organisms under discussion as well as 10 to 20 other coli strains.

Horse serum 221 gave the following results with the 14 cultures under discussion:

	1:100	1:200	1:500	1:1000
1.....	+1	+	1	—
2.....	1	—	—	—
7.....	+	±	1	—
10.....	+	±	1	—
12.....	+	+	1	—

With seven other strains a negative or only slight agglutinin reaction was obtained at 1:20, and with the remaining two a fair and slight reaction at 1:50.

With 23 additional coli cultures from abnormal stools four gave no reaction at 1:20, six showed a tendency to agglutinate at 1:50, eight still showed a tendency at 1:100, four at 1:200; one, Colon Y, still gave a good reaction at 1:500, and showed a tendency at 1:1000. This one organism, Colon Y, has been of especial interest in our laboratory since it was isolated from diarrheal stools from several cases. It conforms absolutely to the rigid cultural tests for *B. coli*, and behaves in its agglutination reactions very much like the Flexner Manila culture of paradysentery. Colon X rabbit serum, while agglutinating its own organism in a dilution of 1:1000, agglutinated strain 2 to 1:100 completely, gave a good reaction with strain 7 at 1:500, no reaction with strains 3 and 4, and only a fair reaction at 1:50 with strains 10:12.

Further attempts to obtain a serum which should be specific for the colon group were then abandoned. We are inclined to the belief that just as there are innumerable coli strains, so there may be as many specific agglutinins, and any attempt to classify coli by agglutination reactions would be correspondingly futile. It would be equally incorrect to refuse to include in the colon group any bacillus because it failed to be agglutinated by a serum which had been obtained from an animal which had received numerous strains of *B. coli*.

## BIOCHEMICAL CHARACTERISTICS.

In addition to the 14 coli cultures mentioned in connection with the agglutination experiments, 34 other strains of coli were studied, the entire number, 48, being obtained from 34 different specimens of stools from as many persons. These 48 strains are, therefore, of intestinal origin and are all members of the colon group even if a very few do not conform strictly to every test for *B. coli*. All produce gas in dextrose broth, all but two are acid producers, and none liquefy gelatin in 10 to 14 days. All but four produce indol in three days, all produce nitrites in nitrate solution, all but five produce gas in neutral red lactose broth. Twelve out of 30 that were tested on saccharose broth gave no gas, or only a bubble, 18 giving gas which varied from 1 to 50 per cent in amount. The gas produced in dextrose broth by these 48 cultures varies from 15 to 90 per cent of the closed arm.

2 gave 15 %	7 gave 40 %	2 gave 60 %
2 " 20	2 " 45	1 " 65
6 " 25	10 " 50	2 " 75
4 " 30	1 " 55	1 " 90
8 " 35		

Of the total gas collected in the closed arm of the fermentation tube  $\text{Co}_2$  formed from a trace to 65 per cent. H was constantly present. The tests of these bacilli, which undoubtedly belong to the human colon group, indicate that different strains produce a widely different amount of gas and, to some extent, of proportion of  $\text{Co}_2$  to H. Fifty colonies having the characteristics of colon bacilli were fished from a lactose litmus agar plate which had been inoculated directly with normal feces from a single case. These 50 cultures were then inoculated into saccharose, lactose, dextrose, and mannite with the following results:

All 50 cultures fermented mannite with the production of acid and of visible gas, coagulated milk within three days, and produced indol within the same time; none of them changed gelatin within a period of 10 days. All changed neutral red to yellow red in lactose broth and caused a gas production of between 20 and 30 per cent of the height of the closed arm of the fermentation tube. The amount of gas in dextrose varied between 20 and



60 per cent, of which  $\text{Co}_2$  composed 20 to 60 per cent. The following division of gas amount was noted: 20 per cent, 20; 25 per cent, 12; 30 per cent, 7; 35 per cent, 4; 40 per cent, 4; 50 per cent, 2; and 60 per cent, 1.

The chief difference was noted in saccharose broth. In this sugar 23 produced visible gas and 27 produced none. Of the 23 producing gas, only 18 produced appreciable acid. Of the 27 producing no gas, 18 produced acid. The gas amount in the acid producing cultures varied from 1 to 10 per cent. In the cultures producing an excess of alkali, 1 to 40 per cent. Of the cultures producing the greatest amount of gas in dextrose and lactose, two produced no visible gas in saccharose.

Of the 98 *B. coli* cultures studied, only nine would be ruled out of the strict *B. coli* type, and of these five conform to every test but that of fermenting lactose, and four to every test except that of indol production. Seven of the nine were obtained from diarrheal stools. In fact, these slightly irregular *coli* types seem to be more frequently encountered in abnormal than in normal stools.

Passing now from the study of colon types as found in the human intestines to the examination of drinking waters suspected of pollution, we see, as has already been shown, that a search for *B. coli* and the sewage streptococcus would hardly fail to reveal pollution. After some preliminary work with the presumptive test for *B. coli* in dextrose broth, which will be discussed again later, we were led to the conclusion, that while the correct percentage of total gas and a proper gas ratio might really indicate the presence of *B. coli*, yet the reverse proposition, that an incorrect percentage of total gas and an improper gas ratio precludes the presence of *B. coli*, cannot be held.

In the examinations of Croton tap water we used the Lawrence method for *B. coli* identification as developed and used by Mr. Gage, and in every case where *B. coli* is spoken of as being found the cultures were fully identified at the laboratory.

The value of the so-called presumptive test for *B. coli*, depending, as it does, on the amount of gas formed in fermentation tubes from dextrose broth and the proportion of carbon dioxide

and hydrogen in the gas formed, would unquestionably be great, if it could be proved to be accurate as well as rapid. Gage (Massachusetts State Board of Health, 1902), however, finds that certain waters of known purity would be condemned by the presumptive test, while in the examination of the shellfish very erroneous results would be obtained, if the presumptive test alone were used.

Prescott and Winslow in their book, "Elements of Water Bacteriology," speak of the value as a presumptive test of the gas formation in dextrose broth. They admit that the distinction between positive and negative is not absolute, but they consider Whipple's results, obtained by examining a number of surface water supplies, as being very striking and in general sound.

In our experience we have found that our own municipal supply, with its main reservoirs and lakes, guarded carefully, and many small streams in the region of Mt. Kisco and Katonah, left comparatively unprotected and open to definite pollution, is never free from coli. So far as we have tested, it is constantly present in amounts of 10 c.c., and frequently in 1 c.c. The filling of the new reservoir is expected to take place in April. This will give longer storage to the water and thus improve its quality. These cultures were isolated by us by the method used by Mr. Gage at the Lawrence Experiment Station. The method, as modified by us, consists in inoculating dextrose broth with varying amounts of water under examination, and plating out on lactose litmus agar from any tubes showing evidence of fermentation after 16:18 hours' growth at 37° C. Thus in 48 hours lactose litmus agar plates will be obtained which, to the trained observer, will give a better basis for a preliminary judgment of the water being tested than the preliminary fermentation test alone, and colonies picked from these lactose litmus agar plates can be transferred to agar and carried through the confirmatory tests for *B. coli* as a matter of routine procedure. We present a table (Table 3), giving in parallel columns the results of *B. coli* cultures isolated from Croton water and confirmed by the Lawrence method, in column 1, and results of presumptive tests given in column 2. The *B. coli* cultures were all isolated from samples taken by

us at the tap in the Research Laboratory, foot of East 16th Street, and a few from the tap at City Hall park. The presumptive tests, on the other hand, were made on samples taken from the 135th Street Gate House. The table has the additional value, even though the results are few in number, of giving under the heading of the presumptive tests the published reports\* of the Water Supply Department, and their opinion of the water was based on Mr. Whipple's provisional interpretation of presumptive tests given out in 1903, yet, while the presumptive tests are almost uniformly negative, *B. coli* has been constantly isolated by us in quantities varying from 1 c.c. to 10 c.c. The Water Supply Department had further stated that considerable purification takes place between 135th Street Gate House and the City Hall. At 16th Street, which is midway between the two stations, we should expect to get water that had at least half the purification that the City Hall water would show and therefore purer than at 195th Street.

TABLE 3.

Date 1904	Coli Cultures Isolated at Research Lab. from Croton Water	Quantity and Sources	Water Supply Reports Based on Presumptive Tests		
Sept. 6.....	Croton 1	1 c.c. E. 16th St.	c.c. 0.1	c.c. 1.0	c.c. 10.0
	" 2		0	0	0
	" 3				
	" 4				
Sept. 13.....	" 8	1 c.c. E. 16th St.	0	0	0
Sept. 20.....	" 9, 10, 11	1 c.c. E. 16th St.	0	0	0
Sept. 28.....	" 13, 14	10 c.c. E. 16th St.	0	0	0
Oct. 4.....	" 15				
	" 18 (City Hall)	10 c.c. E. 16th St.	0	0	0
Oct. 11.....	" 19, 20, 21	1 c.c. E. 16th St.	0	0	0
	" 22, 23	10 c.c. City Hall	0	0	0
Oct. 15.....	" 24, 25	1 c.c. E. 16th St.	0	+	+
	" 26, 27	1 c.c. City Hall			
Nov. 23.....	" 28, 29	1 c.c. E. 16th St.	0	0	0
Nov. 25.....	" 31, 32, 33	1 c.c. "	0	0	0
Nov. 26.....	" 34, 35	100 c.c. "	0	0	0
		(10 c.c. not tested)			
Nov. 28.....	" 36, 37, 38, 39	10 c.c. E. 16th St.	0	0	0
Nov. 29.....	" 40, 41	10 c.c. "	0	0	0
Nov. 30.....	" 42	1 c.c. "	0	0	+
	" 43, 44	10 c.c. "			
Dec. 1.....	" 45, 46	40 c.c. "	0	+	0
Dec. 2.....	" 47, 48	10 c.c. "	0	0	0
Dec. 5.....	" 49, 50, 51, 52	10 c.c. "	0	0	0
Dec. 6.....	" 53, 54	10 c.c. "	0	0	0
Dec. 7.....	" 57, 58, 59, 60	10 c.c. "	0	0	+
Dec. 8.....	" 61, 62	10 c.c. "			
	" 63	10 c.c. "	0	0	+
Dec. 9.....	" 64, 65, 66	10 c.c. "	0	0	0

\* We are indebted to Dr. Jackson, Director of the laboratory, for these reports.

We have repeatedly found that waters giving a positive result with presumptive test in .1 c.c. have given a negative result with 1 c.c. and with 10 c.c., the growth of a considerable number of varieties of bacteria interfering with the action of the colon bacilli.

A presumptive test which frequently fails completely to reveal the presence of *B. coli* where it is a comparatively simple matter to find it by another method is certainly of questionable value, and should be subjected to rigid investigation before basing any very definite opinions on the quality of the water supply under examination.



## THE CHEMICAL PHASES OF A WATER SOFTENING PROBLEM.

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THE application of methods of analysis, and their interpretation with reference to problems of water softening, depend chiefly upon the relative amounts of calcium and magnesium which the water contains. Since the composition of water is in a large measure dependent upon geological conditions, it would not appear to be possible to apply general methods of analysis, or to expect that any one method of interpreting analyses would be universally applicable. In a study of the character of a portion of the water supply of the city of Columbus, Ohio, several analytical features were developed, which illustrate the limitations of general methods of analysis for waters containing considerable quantities of magnesium.

As water softening is probably of growing importance in connection with the hygienic purification of hard waters, it seemed to the writer that an outline of the chemical phases of one of these water-softening problems might be of general interest. The suggested method of stating the results of the analysis of water for softening purposes is proposed with the hope that it may add strength to the plea for the adoption of a procedure for expressing the analytical data necessary to calculate the chemical treatment of a water, which shall be merely a statement of facts analytically determined.

### THE STATUS OF THE MINERAL ANALYSIS OF WATER.

The analysis of water for softening purposes essentially differs from a sanitary analysis, in that it is chiefly concerned with the dissolved inorganic substances which constitute hardness. The relative amounts of calcium and magnesium forming the alkalinity and incrustants are the principal features of an analysis of this character. Computations of the chemicals required to soften a hard water have generally been based upon the assembled

results of a gravimetric analysis of the dissolved mineral constituents. While there can be no question as to the reliability of these data in so far as they relate to the total quantities of calcium, magnesium, and the acid radicals present, respectively, as soon as it becomes a question of deducing from these data the amounts of the specific salts of these bases conceived to exist in solution in the water, such a variety of views exists as to the procedure to be followed that different observers, working upon the same water, rarely report concordant results.

The question of the interpretation of a mineral analysis of water has been the subject under discussion in the more recent papers on water softening, the conclusions of the authors suggesting greater simplicity in expressing results, urging that only the facts of the analysis be reported, and demonstrating that the salt combinations are unnecessary for calculating a softening treatment. Thus McGill,<sup>1</sup> in a paper on "Water Treatment," discusses the vexed question of the mineral analysis, and urges that results be reported in terms of the oxides of the bases, and in terms of the acid radicals present, showing also that these data, supplemented by the determination of the alkalinity and the incrustants, respectively, are sufficient for making calculations of the chemicals required to soften a given water.

Handy,<sup>2</sup> in an article on "Water Softening," suggests the wisdom, in certain cases, of expressing the results as uncombined mineral constituents, thus departing from the older method of reporting the bases and the acid radicals in combination, since the complexity of many waters renders data thus expressed practically matters of conjecture.

A study of the composition of a portion of the water supply of the city of Columbus, Ohio, has shown the applicability to this water, under the conditions existing during the investigation, of a form of expressing the results of its analysis, especially relative to softening, in which the bases alone are reported. As an illustration of the facility with which the treatment of this water may be calculated from an analysis thus expressed, the necessary data

<sup>1</sup> Bull. No. 55 Amer. Ry. Eng. and Maintenance of Way Association.

<sup>2</sup> Proc. Eng. Soc. Western Penna., December, 1903.

and the methods of analysis employed in their determination are now presented in detail.

DATA NECESSARY FOR SOFTENING CALCULATIONS AND METHODS  
USED FOR THEIR DETERMINATION.

To calculate the amounts of chemicals necessary for the treatment of a magnesium water, such as the one under study, the following data are required:

- The free and the half-bound carbonic acid.
- The alkalinity.
- The incrustants.
- The total magnesium.
- The total calcium.
- The incrusting calcium.

The free and the half-bound carbonic acid and the alkalinity were determined by the usual methods; the incrustants by Soda Reagent; the total magnesium by a modification of Pfeifer's method, which consists in precipitating the magnesium with an excess of standard lime water, and determining the excess by titration with standard sulphuric acid; the total calcium by the usual gravimetric method, and also by calculation from the total hardness; the incrusting calcium by a gravimetric determination of the calcium not removed by boiling, correcting for the calcium present due to dissolved normal carbonate.

*The determination of total magnesium.*—Into a porcelain evaporating dish measure 100 c.c. of the water, and add that amount of  $\frac{N}{20}$  sulphuric acid required to neutralize the alkalinity. Boil to a volume of about 50 c.c. to expel the carbonic acid, and transfer the solution to a 200 c.c. flask of Jena glass, graduated at 205 c.c. and provided with a ground glass stopper. Introduce by means of a pipette 25 c.c. of clear saturated lime water. (The amount of calcium oxide present must be 50 per cent in excess of the amount required to precipitate the magnesium.) Quickly make up the volume to 205 c.c. with boiling distilled water, stopper the flask, and mix thoroughly. Place the flask on the water bath for 15 minutes, cool, and allow the precipitate to settle completely. Pipette off 100 c.c. of the clear solution into a 100 c.c. nessler tube containing slightly less  $\frac{N}{50}$  sulphuric acid than

will neutralize the excess of calcium oxide, titrate with  $\frac{N}{50}$  sulphuric acid, with phenolphthalein as the indicator, using the glass stirrer and observing the same precautions as in the Sehler method for the determination of free carbonic acid. Owing to the effect of temperature upon the solubility of calcium oxide, it has been found to be necessary to make a blank determination with distilled water and lime water.

It is clear that in this blank determination the procedure must be identical with that used for the water, and that great care must be exercised to avoid carbonation of the lime water through exposure to the air. Twice the difference between the number of c.c. of  $\frac{N}{50}$  sulphuric acid required to neutralize 100 c.c. of the lime water blank, and the number of c.c. of  $\frac{N}{50}$  sulphuric acid required to neutralize the excess of lime in 100 c.c. of the sample, is an expression, in terms of  $\frac{N}{50}$  sulphuric acid, for the amount of magnesium present in 100 c.c. of the water under examination. The following formula is convenient for calculating the amount of magnesium in parts per million.

Let  $S$  = Number of c.c. of  $\frac{N}{50}$   $H_2SO_4$  required to neutralize 100 c.c. of the lime water blank.

Let  $N$  = Number of c.c. of  $\frac{N}{50}$   $H_2SO_4$  required to neutralize the excess of calcium oxide in 100 c.c. of the mixture of lime water and of hard water.

Then Magnesium (Mg) Parts per million =  $2.4 (2S - 2N)$ .

The method gives very satisfactory results provided that the precautions mentioned above are rigidly observed. The change in the strength of the lime water during the heating involved in this method is illustrated by the following example of an analysis:

Strength of Lime Water (Available CaO).		
Parts per million.		
Lime Water Blank.		
Before Heating		After Heating
974		896
Analysis.		
Formula: $Mg = 2.4 (2S - 2N)$ . = $2.4 (40 - 18)$ therefore		
$S = 20$	$N = 9$	= 53 parts per million.

A gravimetric analysis of the same water gave 55 parts magnesium per million. The above volumetric results agree very closely with the gravimetric results, and appear to be well adapted for the rapid determination of magnesium in hard waters.



*Calculation of the Total Calcium.*—The total hardness of a water expressed in terms of calcium carbonate, is the sum of the alkalinity and the incrustants, which are generally expressed in terms of calcium carbonate. If from the total hardness thus expressed an amount of calcium carbonate equivalent to the magnesium present be deducted, the remainder will, obviously, be an expression in terms of calcium carbonate, for the hardness due to calcium.

*Incrusting Calcium.*—The determination of the incrusting calcium consists in boiling 500 c.c. of the water in a porcelain dish to a volume of about 200 c.c.; removing the precipitated carbonates by filtration and determining the calcium in the filtrate by the usual gravimetric method. The amount of incrusting calcium is the difference between the total amount of calcium found to remain after boiling, and the amount of it due to dissolved normal carbonate of calcium. Comey states that the solubility of the normal carbonates consists of 20 parts calcium carbonate and of 17 parts magnesium carbonate per million. The correction to be applied is, obviously, in terms of calcium eight parts per million. ( $20 \times 0.40$ .)

#### CALCULATION OF THE ANALYSIS.

From the data listed above the analysis may be calculated. The results are expressed under two separate headings, namely, Alkalinity and Incrustants; and, as previously mentioned, the bases are expressed as such.

#### COMPONENT BASES OF THE ALKALINITY.

*Calcium.*—To compute the amount of calcium attributing to the alkalinity, that quantity present as an incrustant is subtracted from the total calcium.

*Magnesium.*—It is obvious that the difference between the alkalinity determined by titration, and that portion found to be due to calcium, must be an amount of calcium carbonate equivalent to the magnesium component of the alkalinity.

#### COMPONENT BASES OF THE INCRUSTANTS.

*Calcium.*—This constituent is determined directly by the method already described.

*Magnesium.*—The incrusting magnesium, by reasoning analogous to that used in the computation of the calcium contributing to the alkalinity, is the difference between the total magnesium and that constituting a part of the alkalinity.

*Total Incrustants by Calculation.*—The total incrustants in the water, expressed in terms of calcium carbonate, are clearly the sum of their component bases, calcium and magnesium, expressed in terms of calcium carbonate.

The following example is given as an illustration of the application of the foregoing procedures:

<i>Constituents</i>	<i>Parts per million</i>
Total Calcium (Ca)	= 98
Total Magnesium (Mg)	= 44
Alkalinity ( $\text{CaCO}_3$ )	= 252
Total Incrustants ( $\text{CaCO}_3$ )	= 174
Incrusting Calcium (Ca)	= 25

*Calculation of the Component Bases of the Alkalinity.*

<i>Calcium</i>	<i>(Parts per million)</i>	<i>Magnesium</i>	
Total Calcium	= 98	Alkalinity	= 252
Incrusting Calcium	= 25	Calcium Component	= 183
Calcium as alkalinity	= 73	Magnesium as $\text{CaCO}_3$	= 69
Equivalent Calcium Carbonate	= 183	Magnesium	= 17

*Calculation of the Component Bases of the Incrustants.*

<i>Calcium</i>	<i>(Parts per million)</i>	<i>Magnesium</i>	
Incrusting calcium (Ca)	= 25	Total magnesium (Mg)	= 44
Equivalent calcium carbonate	= 63	Mg component of alkalinity	= 17
Total incrustants computed	= 176	Incrusting Mg	= 27
Total incrustants by "Soda Reagent"	= 174	Equivalent calcium carbonate	= 113

To facilitate a comparison of this form of expressing an analysis with the older method of reporting data for water softening purposes, the above constituents are shown assembled in the following table:

<i>Alkalinity</i>		<i>Incrustants</i>	
	Ca = 73	Ca = 25	
	Mg = 17	Mg = 27	
Total alkalinity	= 252	Total incrustants	= 176 By "Soda Reagent"
Half-bound carbonic acid	= 111		= 174 By Computation

## THE THEORY OF THE ACTION OF LIME AND SODA ASH AS SOFTENING AGENTS FOR A MAGNESIUM WATER.

Lime and soda ash are of chief economic interest for water softening upon a large scale. The theory of their action upon a magnesium water is as follows:

*Lime.*—As is well known, the softening by lime of hard waters containing calcium alone, consists only in a neutralization of the free and the half-bound carbonic acid present. In the presence of magnesium, however, complications arise owing to the property of magnesium of forming soluble basic salts. For a magnesium water, such as was the water under discussion, a neutralization of the carbonic acid, converts the magnesium component of the alkalinity to a soluble basic carbonate, the removal of which is effected only by an additional amount of lime, which is that quantity sufficient to change the magnesium to the difficultly soluble hydrate. The incrusting magnesium must also be converted to hydrate since by the action of the soda ash subsequently added, this portion also of the magnesium is converted to a basic carbonate requiring an alkali for its precipitation. It is clear that the amount of lime required to soften a magnesium water is an amount sufficient to neutralize the carbonic acid, together with the additional amount necessary to convert to the hydrate form, the total quantity of magnesium present.

*Soda ash.*—Soda ash, as is clearly understood, precipitates the calcium component of the incrustants, and converts to a basic carbonate the magnesium component. Since the action of lime upon the incrusting magnesium results in the formation of an equivalent amount of a calcium incrustant, the bases of the incrustants may be considered as consisting of calcium alone, so that, in calculating the amount of soda ash required to treat a magnesium water similar to the one under consideration, the total amount of the incrustants may be taken as a basis.

It is apparent that magnesium requires for its removal from a hard water, an amount of lime twice as great as would an equivalent amount of calcium. And further, that to compute the amount of lime required, it is necessary to determine only the total amount of magnesium present in addition to the determination of the carbonic acid.

## CONCLUSIONS.

The study of the problem of softening a hard water containing an unusually large relative amount of magnesium, has shown that the data required to calculate the quantities of lime and of soda ash necessary to soften it may be obtained without making a complete mineral analysis. The data are: The carbonic acid, the total magnesium and the incrustants. From the quantities of these constituents in the water, together with a determination of the amount of calcium contributing to the incrustants, there may be calculated the component bases of the alkalinity and the incrustants, respectively.

An assembled analysis of the water is readily computed from these data, and in the opinion of the writer, the statement of the analysis in terms of the bases present, is in sufficient detail. Such a method, obviously, avoids all assumptions as to the specific combinations of the bases and of the acid radicals present. A determination of the quantity of sulphuric anhydrid in the water is unnecessary when the calculation of the softening treatment is based on these data, and therefore the much disputed question of the apportionment of the sulphates and the bases would not appear to be a factor in water softening analyses, at least for conditions similar to those under discussion.

The writer wishes to acknowledge his obligations to Mr. George W. Fuller for invaluable advice and criticisms, and to Mr. George A. Johnson, under whose supervision the investigation was conducted, and with whose valuable counsel and cooperation this paper has been prepared.



## TYPHOID-LIKE BACILLI IN THE WATER SUPPLY OF FREDERICTON, N. B.

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THE City of Fredericton, N. B., is situated on the south bank of the St. John River, which at this point is about three quarters of a mile wide, with little current. The tide does not come further up the river than Gagetown, about 35 miles below Fredericton, but the effect of the tide is felt at Fredericton, there being a rise of an inch at high water and five or six inches at low water.

The city is built on sandy soil with here and there layers or banks of clay. By digging down a few feet, water may be reached at any time in the year, and the water level rises and falls according as the season is wet or dry. Every spring many cellars are flooded.

The city has no system of sewerage; but to some slight extent the drains are used as sewers. These flow into the river at different points along the city front. Nearly every house has one or more cess pools. The hospital is situated 100 feet above the pumping station and 300 feet from the bank of the river. This bank is about 20 feet high and there is a gradual incline from the hospital to the edge of the bank. Formerly, there was a sewer from the hospital to a place below the intake; this, however, became plugged and for over a year past the drainage from the hospital has emptied into a hole just behind the hospital and from this receptacle it has drained into the river.

The water supply for the city is taken from the St. John River, 150 feet from the shore and directly opposite the pumping station.

The number of cases of Typhoid fever officially reported in Fredericton (population 7,000) during the last few years are as follows:

The year ending October 31, 1899, 71 cases.					
"	"	"	"	"	1900, 21 "
"	"	"	"	"	1901, 10 "

The year ending October 31, 1902, 33 cases.

“ “ “ “ “ 1903, 29 “

and the number of cases from November 1, 1903, to April 4, 1904, was 24. At the time of writing, there are 15 cases in the hospital and over 50 in the city. Besides the cases which are officially reported, the writer is informed that there are dozens each year which are cared for at home, and are not reported. The cases are from all parts of the city supplied with city water. Across the river at St. Mary's, where artesian well waters are used, there have been no cases of typhoid this year and none for several years past, except one or two and these among those who work in Fredericton and drink Fredericton water. From a careful examination of the distribution of the cases, dissemination through infected milk from one or more milk dealers may be excluded.

On March 15, 1904, the writer received from Fredericton two bottles of water, well packed and in good condition. One of these samples was taken from the river at a point one mile above the city. The second sample was made up of water from running taps in three different parts of the city.

The chemical analyses of these waters were as follows:

	River Water (parts per million)	City Water
Solids at 212° F.....	8.	72.
Solids after ignition .....	3.	32.
Loss on ignition.....	5.	40.
Free ammonia.....	0.09	0.015
Albuminoid .....	0.045	0.1875
Nitrogen in Nitrates and Nitrites .....	0.111	0.267
Phosphates .....	free	free
Chlorine .....	1.0	30.

The above analyses were made by Mr. F. T. Shutt, Chemist of the Experimental Farm at Ottawa. Commenting on the above analyses, he stated that "It is to be regarded as a suspicious sign that the differences in the analytical data are to be observed, and that between the point where the sample was taken in the river and the mouth of the intake pipe the quality of the water was, for some cause, changed for the worse."

The bacteriological analyses were as follows:

*River Water:*

Total number of bacteria per c.c., 141,000.

Liquefying colonies chiefly  $\frac{\text{B. fluorescens liquefaciens}}{9000 \text{ per c.c.}}$ .

Non-liquefying colonies, 132,000 per c.c.

Fermentation tubes containing two per cent glucose broth inoculated with  $\frac{1}{4}$  c.c. of water gave no growth in the closed arm after 48 hours at 37° C.

Inoculated with  $\frac{1}{2}$  c.c. water gave slight growth and cloudiness and seven per cent gas in 48 hours.

Carbolic broth (containing four c.c. of five per cent solution of carbolic acid in 100 c.c. of beef broth) inoculated with  $\frac{1}{4}$  c.c. of river water and incubated at 37° C. gave no growth in 48 hours. Inoculated with  $\frac{1}{2}$  c.c. water and incubated for 48 hours at 37° C., no growth.

Two guinea pigs were inoculated. One, intraperitoneally, with two c.c. of glucose broth from fermentation tube inoculated with  $\frac{1}{2}$  c.c. water; and the other, intraperitoneally, with two c.c. of the carbolic broth culture inoculated with  $\frac{1}{2}$  c.c. water. Both cultures had previously been incubated for 48 hours at 37° C.

The results were nil; the animals remained well and healthy.

*City water:*

Total number of bacteria per c.c., 34,650.

Liquefying colonies, principally  $\frac{\text{B. fluorescens liquefaciens}}{3150 \text{ per c.c.}}$ .

Non-liquefying colonies, 31,500 per c.c.

Of these, a number looked like colonies of the colon bacillus.

Agar plates incubated at 37° C. for 24 hours gave 16,150 colonies of bacteria per c.c. Colon bacilli were present in considerable numbers.

Fermentation tubes inoculated with  $\frac{1}{4}$  c.c. water and incubated at 37° C. gave 20 per cent gas in 48 hours. Others inoculated with  $\frac{1}{2}$  c.c. water gave 45 per cent gas in 48 hours.

Carbolic broth tubes inoculated with  $\frac{1}{4}$  c. c. water and incubated at 37° gave considerable cloudiness in 24 hours.

Gelatin plates were made from the carbolic broth cultures

and these plates gave practically a pure culture of colon-like bacteria. These plates were carefully examined and 21 colonies, which seemed to resemble the growth of *B. typhosus* on gelatin, were isolated and these sub-cultures were passed through a number of differential tests to see if they resembled the typhoid or the colon bacillus. These tests will be given in detail later on.

*Animal inoculations.*—Two guinea pigs were inoculated with cultures obtained from the tap water.

One guinea pig was inoculated intraperitoneally with two c. c. of a 36-hour old glucose broth culture inoculated with  $\frac{1}{2}$  c. c. water. Eight hours afterwards the animal appeared drowsy, with labored breathing, the hind legs were partly paralyzed, general malaise, and the animal died 24 hours later.

Postmortem made directly after death showed fibrinous serous peritonitis. Spleen and heart-blood contained bacteria resembling *B. coli*.

The other guinea pig, injected intraperitoneally with two c. c. of a 36-hour old carbolic broth culture inoculated with  $\frac{1}{2}$  c. c. tap water showed the same symptoms as the animal inoculated with the glucose broth culture. It died in 36 hours. Postmortem appearances similar to above animal.

The 21 colonies isolated from gelatin plates, inoculated with carbolic broth, were put through a number of differential tests and compared with typical cultures of *B. typhosus* and *B. coli*. The results of these tests are given in tabular form, and a few explanatory remarks are necessary in connection therewith.

The surface colonies selected from the gelatin plates were those which grew more slowly and were thinner than the majority of the colonies on the plate; many of which showed the typical vine leaf markings and thicker and more rapid growth of *B. coli*.

*Dextrose Broth.*—This broth was made up from Liebig's extract of beef, one per cent of peptone and two per cent of dextrose. *B. coli* gave from 50 per cent to 75 per cent of gas in this medium. Nos. 1, 3, 4, 6, 10, 12, 14, 17, 19, and 20 produced gas in this medium, varying from 10 to 60 per cent.

*Indol.*—Dunham's solution was employed. Cultures 5 and 10 days old, incubated at 37° C., were used for this test. The typical



*B. coli* produced indol in abundance. There was no formation of indol by the other varieties.

*Milk*.—The milk cultures were incubated at 37° C., and held under observation for a month at this temperature. Nos. 4, 14, 16, and 20 coagulated. Sixteen and 20 produced considerable gas.

*Potato*.—*B. coli* produced a thick, yellowish-brown growth on the potatoes used, and numbers 1, 3, 4, 6, 9, 10, 12, 14, 17, 19, 20, and 21 also gave colored growths, in some cases not so marked in color or thickness as *B. coli*. The growths on Nos. 5 and 11 were very slightly tinged with color. There was a thin, transparent growth, scarcely visible to the naked eye, upon the surface of the other potatoes. These growths were submitted to a microscopical examination which showed the presence of large numbers of bacteria.

*Litmus Whey, containing one per cent of peptone*.—Cultures were incubated at 37° C. for 10 days. Reaction was tested on the 5th and on the 10th days. Nos. 4, 6, 14, 16, and 20 showed marked acidity. Most of them taking 30 per cent of  $\frac{N}{10}$  alkali to neutralize. The remaining cultures wanted less than five per cent of  $\frac{N}{10}$  alkali to neutralize the acidity.

Those varieties which showed most resemblance to *B. typhosus* were put through a few more differential tests with the following results:

*Neutral Red Glucose Agar*.—*B. coli* produced gas and a canary yellow fluorescence, and the remaining cultures showed growth, but no change in color and no production of gas.

*Proskauer and Capaldi's Medium, No. I*.—A culture of *B. typhosus* produced no growth or change in reaction in this medium. *B. coli* gave good growth with acid reaction. Nos. 1, 2, 7, 15, and 18 gave slight growth with acid reaction. Nos. 13 and 19 gave growth with acid reaction later changing to an alkaline reaction.

*Proskauer and Capaldi's Medium, No. II*.—Nos. 1, 2, 6, 7, 13, 15, 16 and 18 gave growth with acid reaction, resembling growth of the *B. typhosus* in this medium. *B. coli* gave growth with a neutral or faintly alkaline reaction of this medium.

*Agglutination.*—The antityphoid serum used for this was obtained through the courtesy of the Parke, Davis Company, of Detroit. It was a very powerful serum. The agglutinating limit of this serum with my *B. typhosus* was not worked out. In a dilution of 1:250,000, it gave a marked agglutination with this culture. For the purpose of testing the various varieties, four dilutions were made up—1:100,000, 1:10,000, 1:1,000, and 1:500. Four varieties, Nos. 8, 13, 15 and 18, gave complete agglutination in dilutions of 1:1,000 and 1:500, No. 2 gave complete agglutination with dilution 1:500.

A glance at these results will show that a number of these varieties were very closely allied to *B. typhosus* and showed greater resemblance to this organism than to the varieties described by Houston and Horrocks under the name of *B. typhosus simulans*. On the Proskauer and Capaldi's medium 1, the growth compared with *B. typhosus* was atypical.

A guinea pig inoculated with  $\frac{1}{2}$  of an agar culture of variety No. 18 incubated for 24 hours at 37° died in 36 hours. The bacteria present in the spleen and heart blood were similar to the organism inoculated.

It seems unnecessary to comment upon the condition of the water supply of Fredericton. The drainage of the city and some of its sewage, the sewage from the hospital and the drainage from the cemetery, all empty into the St. John River close to the shore and these sources of contamination are both above and below the water intake and there seems to be sufficient tide in the river to contaminate the water *above* the intake, with the sewage which enters the river from *below* the pumping station.

NOTE.—The media used in this work were, unless otherwise stated, prepared according to the recommendations of the Laboratory Committee of the American Public Health Association.

All cultural tests were made in duplicate, some in triplicate.



## THE PERSISTENCE OF AGGLUTINABILITY IN TYPHOID BACILLI IN WATER.

EDWIN O. JORDAN.

It has been established by the researches of Bail,<sup>1</sup> Walker<sup>2</sup> and Müller<sup>3</sup> that the agglutinability of typhoid bacilli can be altered by gradual immunization to immune serum. Kirstein<sup>4</sup> has also shown that it is possible to effect a slight increase or decrease in the agglutinability of certain races by subjecting the bacilli to various chemical and physical influences; change in agglutinability, however, was not great and a permanently inagglutinable race could not be produced.

The question of a possible loss of agglutinability in typhoid bacilli in sewage or water is one of practical importance, since in many instances recognition and identification of the typhoid bacillus have been made to hinge upon the positive outcome of the agglutination reaction.

Both observation and experiment bear upon this question. In the former category may be placed the observations of Remlinger and Schneider<sup>5</sup> who found in water, soil and other situations bacteria that were said to resemble typhoid bacilli in every respect except that they failed to agglutinate with typhoid serum and were not pathogenic for animals. Remlinger and Schneider were tempted to conclude that these were typhoid germs that had parted with their agglutinability under the conditions to which they had been subjected.

Dealing with the same matter on the experimental side, Remy<sup>6</sup> rejects the view advanced by Wathelet<sup>7</sup> and Grimbert<sup>8</sup> to explain the failure to isolate *B. typhosus* after a more or less prolonged association with *B. coli*. Wathelet and Grimbert believed that the typhoid bacillus, when sown together with *B. coli* "succumbs

<sup>1</sup> *Archiv f. Hyg.*, 1902, 42, p. 307.

<sup>3</sup> *Münch. med. Wchschr.*, 1903, 50, p. 56.

<sup>2</sup> *Jour. of Path. and Bact.*, 1902, 8, p. 34.

<sup>4</sup> *Ztschr. f. Hyg.*, 1904, 46, p. 229.

<sup>5</sup> *Ann. de l'Inst. Past.*, 1897, 11, p. 55.

<sup>6</sup> *Ibid.*, 1900, 14, p. 705.

<sup>7</sup> *Ibid.*, 1895, 9, p. 252.

<sup>8</sup> *Compt. Rendu de la Soc. de Biol.*, 1894, 46, p. 399.



in the struggle." Remy maintains rather that the properties of the two organisms are profoundly modified by life in common. As one consequence of this association the typhoid bacillus, according to Remy's statements, may lose its sensibility towards the specific agglutinin. When grown separately in neutral peptone water both organisms retained their specific qualities. Horrocks<sup>1</sup> on the other hand, in a similar experiment, found that *B. typhosus* when grown with *B. coli* maintained its power of agglutinating with specific serum as long as it could be isolated from the mixture (13 days).

The observations of Remy and Horrocks thus stand in direct conflict. The practical importance of accumulating facts as to the frequency with which so significant a differential peculiarity as agglutination is destroyed by aquatic life or by association with *B. coli* leads the writer to record a few experiments on this point made in connection with a study of the longevity of typhoid bacilli in water.<sup>2</sup>

*Experiment 1.*—Two loopfuls of a 24-hour old agar culture of *B. typhosus* (Strain II) and one loopful of *B. coli* (Strain I) were introduced into 500 c.c. of sterile tap water in a glass flask and kept at room temperature for 12 days. Typhoid bacilli were then isolated by plating in litmus lactose agar, no difficulty being experienced in discovering typhoid colonies. Agglutination was tested macroscopically with immune serum from a rabbit, using the stock typhoid culture (Strain II) as a control. Both the stock culture and the culture obtained from the tap water agglutinated to exactly the same degree (1:500) with the typhoid serum. The colon bacilli that had been kept in the water with typhoid bacilli showed no agglutination whatever with a 1:40 dilution of the typhoid immune serum.

*Result.*—No change in agglutinability of the typhoid bacillus after association with *B. coli* in tap water for twelve days.

*Experiment 2.*—The conditions of this experiment were similar to those described in Experiment 1. The immune serum used in this instance agglutinated completely in 1:1000 dilution. The typhoid kept in stock (on agar) and the culture isolated after 18 days' sojourn in water together with *B. coli*, agglutinated in precisely the same way in a 1:200, 1:400, 1:1000 and 1:2000 dilution.

*Result.*—No change in agglutinability after association with *B. coli* in tap water for 18 days.

*Experiment 3.*—Five hundred centimetres of sterile sewage was inoculated with a mixture of *B. typhosus* and *B. coli* in the proportions given in Experiment 1. Immune rabbit serum agglutinating completely in 1:1000 dilution

<sup>1</sup> *Bacteriological Examination of Water*, 1901, p. 219.

<sup>2</sup> *Jour. Inf. Dis.*, 1904, 1, p. 641.

was used. Twenty days after inoculation *B. typhosus* was isolated from the mixture by the plate method of Hiss. This culture agglutinated exactly like the stock culture, when compared in 1:1000 dilution. The culture of *B. coli* derived from the mixture showed no agglutination in a 1:40 dilution of the serum.\* Another flask of sewage was inoculated at the same time with a pure culture of *B. typhosus* and the organisms recovered after 20 days. In this case also, agglutinability was normal.

*Result.*—When inoculated into sterile sewage both alone and in association with *B. coli*, the typhoid bacillus retained its agglutinability unimpaired for 20 days.

*Experiment 4.*—Two loopfuls of a 24-hour old agar culture of *B. typhosus* (Strain X<sup>1</sup>) and one loopful of *B. coli* (Strain IV) were introduced into 500 c.c. of sterile tap water in a glass flask and kept at room temperature. *B. typhosus* was isolated from the mixture after 34 days and was agglutinated by immune serum (Strain II) in 1:1000 dilution precisely like the control culture. *B. coli* from this flask showed no agglutination in 1:40 dilution of the serum.

*Result.*—No change in agglutinability after association with *B. coli* in tap water for 34 days.

From these experiments it appears (1) That the typhoid bacillus may be isolated without special difficulty after association with *B. coli* in tap water and sewage for as long as 34 days; (2) That under these conditions some strains of *B. typhosus* retain their property of agglutinability absolutely intact.

\*In some interesting observations recorded by Horrocks (*Bacteriological Examination of Water*, 1901, p. 227) it was found that the varieties of *B. coli* isolated from typhoid dejecta showed much greater sensibility to agglutination with typhoid immune serum than the varieties of *B. coli* found in normal stools. If these results of Horrocks are confirmed they present some perplexing theoretical problems. Horrocks himself in subsequent experiments found that *B. coli* grown in the presence of "typhoid toxins and agglutinins" evinced no increased sensibility to agglutination with typhoid serum. In our own experiments, just cited, *B. coli* after association in water with *B. typhosus* showed no enhanced susceptibility to agglutination with typhoid serum. The observations of Horrocks do not necessarily require the interpretation that he has placed upon them.

<sup>1</sup> *Jour. Infect. Dis.*, 1904, 1, p. 643.

# THE CHEMICAL AND BACTERIAL COMPOSITION OF THE SEWAGE DISCHARGED INTO BOSTON HARBOR FROM THE SOUTH METROPOLITAN DISTRICT.

## WITH SPECIAL REFERENCE TO DIURNAL AND SEASONAL VARIATIONS.

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### INTRODUCTION.

IN any problem of sewage disposal the composition of the sewage to be treated is the first and most essential factor. A few haphazard analyses are not sufficient. Diurnal and seasonal variations must be determined and the total amount of organic matter to be handled estimated with some degree of accuracy. At the Sanitary Research Laboratory of the Institute of Technology we have based our ideas of the efficiency of our various experimental filters upon the studies outlined below—studies which included daily analyses extending over a period of nearly a year, and six special 24 series of hourly samples. With the exception of the Lawrence results, no fuller set of analyses has probably been made for any American city. We have thought therefore that a somewhat full discussion of the data might prove suggestive as to methods of approach, as well as of absolute value in defining the character of the Boston sewage studied.

### STATISTICS OF CONTRIBUTORY AREA.

The entire metropolitan district of eastern Massachusetts covers nearly 200 square miles of territory and includes at present some 24 separate cities and towns. The sewage from this large area is discharged by two main sewers into Boston harbor, the region north of the Charles discharging continuously off Deer Island, while the sewage of the South Metropolitan District is stored in tanks at Moon Island and discharged into the harbor on the

ebb tide. (See map.) A third main sewer is now under construction which will relieve the Moon Island outlet, carrying the flow by gravity from the higher parts of the South Metropolitan District to a point of discharge farther out in the harbor. The South Metropolitan District, as at present constituted, includes the city of Boston (with the exception of East Boston, Charlestown, and the harbor islands); the cities of Newton, Waltham, and Quincy, and the towns of Brookline, Watertown, Milton, Hyde Park, and Dedham.

One large metropolitan interceptor receives the sewage from Newton, Waltham, Brookline, and Watertown, and another drains Milton, Quincy, Hyde Park, and Dedham. Both discharge into a main trunk sewer which receives directly most of the sewage of the city of Boston. Statistics for the metropolitan district as given in the reports of the Metropolitan Water and Sewerage Board omit all discharges entering the trunk sewer itself and not passing through one of the two metropolitan interceptors; but we have collected in Table 1 the data for the whole sewerage system discharging at Moon Island, combining with the figures given in the second annual report of the Metropolitan Water and Sewerage Board of January 1, 1903, statistics obtained from the office of the sewer division of the street department of the city of Boston, through the courtesy of Mr. E. S. Dorr, engineer in charge. The present total population (1903), is calculated by geometrical ratio from the censuses of 1895 and 1900; and the contributing population is estimated by assuming that it bears to the total population the same relation which the number of connections bears to the total number of dwelling-houses as stated in the assessor's records.

The Sewage Experiment Station of the Massachusetts Institute of Technology is situated at 786 Albany Street, Boston, at the point indicated by a star on the map. A  $2\frac{1}{2}$  inch pipe connects the station with the trunk sewer of the South Metropolitan District just below the junction of the Albany Street interceptor and above the junctions of the South Boston and Dorchester intercepting sewers, into which not only those districts and a small portion of West Roxbury, but Milton, Hyde Park, Dedham,







TABLE 1.

STATISTICS OF THE SOUTH METROPOLITAN SYSTEM DISCHARGING AT MOON ISLAND.

Districts	Miles of Local Sewer Connected	Area Served Sq. Mi.	Number of Connections with Local Sewers	Estimated Present Total Population	Estimated Population Now Contributing Sewage	Estimated Number of Persons Served by Each House Connection
Boston—						
Wards 6, 7, 8, 9, 10, 11, 12..	64.18	3.60	15,562	169,200	169,200	10.8
South Boston—						
Wards 13, 14, 15.....	45.52	1.92	5,267	64,700	47,830	9.1
Roxbury—						
Wards 17, 18, 19, 21 (22) ..	96.76	3.79	6,731	121,900	63,570	9.4
West Roxbury—						
Wards (22), 23.....	85.80	12.49	3,356	41,400	24,730	7.4
Dorchester—						
Wards 16, 20, 24.....	185.15	8.64	8,845	98,400	70,360	8.0
Brighton—						
Ward 25.....	59.60	4.28	2,405	22,400	14,350	6.0
Brookline.....	53.61	6.81	2,758	23,000	19,000	6.9
Newton.....	94.85	18.03	4,569	37,200	26,900	5.9
Watertown.....	30.41	4.04	1,467	10,700	7,900	5.4
Waltham.....	38.25	13.63	2,594	25,500	22,050	8.5
Milton.....	3.39	12.59	78	7,300	430	5.5
Hyde Park.....	17.11	4.57	609	14,100	5,360	8.8
Dedham.....	3.32	9.40	17	7,200	95	5.0
Quincy.....	35.93	12.56	952	26,500	5,300	5.6
Totals.....	813.88	116.35	55,210	669,500	577,065	8.1

TABLE 2.

STATISTICS OF THE AREA ABOVE THE SEWAGE EXPERIMENT STATION.

Districts	Miles of Local Sewer Connected	Area Served	Number of Connections with Local Sewers	Estimated Present Total Population	Estimated Population Now Contributing Sewage	Estimated Number of Persons Served by Each House Connection
Boston—						
Wards 6, 7, 8, 9, 10, 11, 12..	64.18	3.60	15,562	169,200	169,200	10.8
Roxbury—						
Wards 17, 18, 19, 21 (22) ..	96.76	3.79	6,731	121,900	63,570	9.4
West Roxbury—						
Wards (22), 23.....	81.88	3.57	3,221	39,800	23,800	7.4
Brighton.....	59.60	4.28	2,405	22,400	14,350	6.0
Brookline.....	53.61	6.81	2,758	23,000	19,000	6.9
Newton.....	94.85	18.03	4,569	37,200	26,900	5.9
Watertown.....	30.41	4.04	1,467	10,700	7,900	5.4
Waltham.....	38.25	13.63	2,594	25,500	22,050	8.5
Totals.....	519.54	57.75	39,407	449,700	346,770	8.9

and Quincy discharge. The statistics for the area contributing the sewage actually analyzed are shown in Table 2. The population served appears to be about 350,000, a little more than one-half the total for the South Metropolitan District.

#### CALCULATION OF THE FLOW.

The first question to be settled concerned the variations in the sewage to be treated at different hours of the day and night; and in order to measure this factor we have made six sets of hourly analyses at different seasons of the year and under various weather conditions. In order to obtain an average analysis of the sewage flowing for 24 hours either one of two methods may be employed. Samples may be taken at short intervals, the volume of each sample being proportionate to the amount of sewage flowing at the time. Then if all the samples be mixed the result is a true average of the whole flow. A second and more convenient method is to take samples at regular intervals during the test and analyze each one separately. Then it is possible to calculate a "weighted average" by multiplying each analysis by the flow at the time of sampling, adding up the results and dividing by the total flow. Either method necessitates some knowledge of the hourly variation in the sewage flow.

We were unable to make meter measurements of the flow of sewage during the 24-hour runs, and it was impossible to make other gaugings on account of the size and the flat grade of the sewer. We were therefore forced to fall back upon indirect computations, which, though rough, possess sufficient comparative value for the weighting of hourly analyses.

The total length of the sewer between our intake and the pumping station at Dorchester is 11,514 feet. The sewer is circular in section, the diameter of the upper 6,874 being 9 feet, and of the remainder 10 feet 6 inches; it was built to a uniform slope of 1 in 2,500. At the point at which the sewer is enlarged from 9 feet to 10 feet 6 inches in diameter there enters from the north the South Boston interceptor, a sewer of circular section 6 feet in diameter, and at a point about 1,000 feet below this there enters from the south the Dorchester interceptor of



5 feet circular section. In the office of the Boston Main Drainage Works, on Massachusetts Avenue, directly opposite the point at which our intake enters the sewer, is installed an automatic gauge which records upon a revolving drum the elevation of the sewage. The pen of this indicator is operated by a float in a well, the latter being connected by a six-inch pipe with the sewer. At the pumping station in Dorchester a similar gauge is in operation at a point just above the screens. The permanent records of these gauges are preserved by the Sewer Division, to whose courteous officials we are indebted for their use and for other valuable assistance. We have also been allowed to examine and make use of the pump-log kept at the Dorchester station. We have therefore, for use in calculating the flow past our intake during any hour, the total amount pumped during that time, and the depth of the sewage at each end of the system at the beginning and end of the hour.

Considering the system below us as a reservoir we have a known amount flowing out (the amount pumped), and a known change in level from which we can calculate the amount flowing in. The calculation is complicated by the presence of the two intercepting sewers previously mentioned which enter the system below us; but one of these enters at too high a grade to affect the storage capacity, and the other stands full for too long a distance back materially to influence it. We shall therefore use in calculating our weighted analyses of sewage for 24 hours the total flow in the sewer as measured by the pump records, corrected for the storage in the system as indicated by the change in level. In comparing our monthly averages of daily analyses with one another we shall multiply each set by the total flow for the month.

In calculating the hourly flow we have first taken the recorded depth at each end of the system at the beginning and at the end of the hour, and calculated the volume of the layer of sewage included between these two surfaces. We have assumed the surfaces to be plane and taken as the cross-section of the layer of sewage the cross-section of the middle-point. The whole volume had to be divided into two parts, one in the nine-foot sewer and the other in the ten-foot-six sewer. The maximum observed

hourly variation in the amount of sewage in the sewer was but 10 per cent of the amount pumped in one hour and was generally less than two per cent. It was therefore necessary to calculate the storage value with an accuracy of only 10 per cent to avoid

TABLE 3.  
FLOW OF SEWAGE—MILLION GALLONS PER HOUR.

TIME	July 1903 22 23	Aug. 1903 13 14	Nov. 1903 16 17	Dec. 1903 22 23	Feb. 1904 17 18	April 1904 12 13
9-10 A. M.	.....	.....	5.65	6.54	6.06	4.57
10-11 "	5.64	4.86	6.20	5.35	5.96	4.57
11-12 "	5.63	4.57	5.98	4.57	4.45	4.57
12- 1 P. M.	5.78	4.86	5.96	4.27	4.45	4.57 4.57
1- 2 "	5.65	4.71	5.96	4.57	5.96	4.57 4.57
2- 3 "	5.64	4.66	5.96	5.96	5.96	4.57 4.57
3- 4 "	5.44	4.83	5.96	5.96	5.96	4.57 4.57
4- 5 "	5.42	4.78	6.38	5.96	5.96	4.57
5- 6 "	5.42	4.68	6.07	5.96	5.96	4.57
6- 7 "	5.66	4.27	5.96	5.96	5.96	4.57
7- 8 "	7.23	4.53	5.96	5.96	5.96	4.57
8- 9 "	5.96	4.40	5.96	5.96	5.96	4.57
9-10 "	5.96	4.37	5.96	5.93	5.96	4.57
10-11 "	5.96	4.31	5.96	5.93	5.96	4.57
11-12 "	5.96	3.88	5.96	5.93	5.96	4.57
12- 1 A. M.	5.96	4.57	5.96	5.93	5.96	4.57
1- 2 "	5.96	4.46	5.96	5.65	5.96	4.57
2- 3 "	5.55	4.45	5.96	5.65	5.96	4.57
3- 4 "	5.55	4.57	5.96	5.85	5.92	4.57
4- 5 "	5.46	4.45	5.96	5.85	5.92	4.57
5- 6 "	5.83	4.50	5.96	5.85	5.92	4.57
6- 7 "	3.35	4.69	5.96	5.85	5.91	4.57
7- 8 "	4.86	4.45	5.96	5.85	5.90	4.57
8- 9 "	4.96	4.84	5.96	6.37	6.04	4.57

an error in the final result greater than one per cent. These calculated changes in the volume of the sewage from hour to hour were then applied as corrections to the quantities pumped as calculated from the pump records. If the height of sewage increased during the hour the correction was added; if it decreased, the correction was subtracted. In Table 3 are given the resulting hourly total flows into the system for the six days on which

we make our tests. They represent the flow past our intake plus the amount of sewage entering the system by the two intercepting sewers previously mentioned.

With regard to the absolute amount of sewage flowing past Albany Street the following data are available:

Messrs. R. G. Hartshorne and L. T. Howard, M. I. T., '04, carried out as a graduating thesis a study entitled "Measurement of the Flow in the Main Intercepting Sewer of Boston." This

TABLE 4.  
FLOW OF SEWAGE IN MOON ISLAND SEWER AT ALBANY STREET.  
(Hartshorne and Howard).

DATE	TIME	MEASUREMENTS			PUMP RECORDS CUBIC FEET PER SECOND	RATIO
		Mean Velocity	Area	Cubic Feet Per Second		
P.M.						
April 18.....	1:53- 2:50	2.04	57.2	116.8	222.9	.52
18.....	2:55- 3:51	2.12	58.6	124.0	222.9	.55
20.....	3:00- 4:14	1.93	63.6	122.8	225.4	.54
25.....	2:17- 3:26	2.17	55.3	120.0	226.9	.53
25.....	3:34- 4:37	2.11	55.6	117.0	226.9	.52
A.M.						
May 5.....	8:54- 9:46	1.41	63.6	89.9	164.7	.55
5.....	9:56-10:45	1.60	63.6	101.7	165.0	.62
14.....	7:40- 7:59	3.20	37.3	119.3	222.4	.54
14.....	8:04- 8:31	3.26	38.2	124.5	218.6—227.9	.54
14.....	8:47- 9:04	3.38	40.7	131.8	228.3	.60
						55.1

investigation was made under the direction of the late Mr. K. S. Sweet, Instructor in Civil Engineering, whose untimely death we, who knew him, still mourn. Messrs. Hartshorne and Howard have permitted us to use their figures comparing the results of meter measurements made in the sewer at a point just below our intake with the total amount pumped as obtained from the pump-logs of the sewer division. In making these measurements a large Haskell meter was used, with a long pitch wheel and electrical connections with a buzzer. A system of multiple point measurements was adopted. By use of a stop watch the time required to make a certain number of revolutions, either 10 or 20, was noted. In this way any clogging of the meter could be

instantly detected and remedied. Eight per cent was the maximum error allowed for in the calculation, but the actual error was undoubtedly much less. The following table, based upon the figures obtained, is calculated to show the ratio between the flow past our intake and the total amount pumped.

The results shown in Table 4 indicate a remarkably constant ratio between the amount flowing at Albany Street and the pumpage at the end of the sewer, a ratio of 50 to 60 per cent. The residue must be accounted for by slip of the pumps and by the contribution of the lower intercepting sewers. The concordance of the results obtained in the measurements seem to us to warrant the assumption that the sewage flowing by Albany Street represents from 50 to 60 per cent of the total entering the Moon Island sewer. At any rate the contribution of our sewer appears to be a constant factor of the whole.

#### METHODS OF SAMPLING AND ANALYSIS.

In the first (July, 1903) series of analyses samples were taken from the petcock of the pump, but the difficulty of obtaining anything like a representative sample in this way is very great. In the later runs our pump was run continuously for the 24 hours, pumping at a rate of about 1500 gallons per hour. Some two-thirds of this amount was wasted, the remainder being delivered into a tank of 540 gallons capacity at such a rate that it was nearly filled in one hour. At each hour the sewage was diverted into a second tank, that in the first tank was thoroughly mixed by stirring, and a half gallon sample bottle was filled from the tank by means of a cock at its mid-depth. The bottle was filled rather slowly and the sewage kept well stirred during the operation. Each sample therefore represents very closely the average composition of the sewage flowing during one hour. The advantage of this procedure is clearly indicated by the smoothness of the curves on diagrams II-VI as compared with diagram I. Analyses were made immediately after the collection of the sample.

The following determinations were carried out on each sample: free and albuminoid ammonia, nitrites, oxygen consumed, chlorine



and dissolved oxygen. The samples were also examined bacteriologically and the following groups of organisms counted; on lactose gelatin at 20° C. the total number of organisms, the number of liquefiers and of acid formers; on lactose agar at 37° the total number and the number of acid formers; and on agar at 20° the number of facultative anaërobes.

The chemical analyses were made according to the methods now generally employed in this country as described for example by Richards and Woodman (1904.) We have used permanent standards for the ammonia readings as suggested by Jackson (1900). All ammonia and nitrite values are reported in terms of nitrogen, and all the chemical results are expressed in parts per million. We have adopted the plan of reporting the dissolved oxygen in parts rather than in per cent of saturation. While the latter method of expression may have had some significance in the study of surface waters it has no value whatever in connection with sewage work or with filtration work in general. Owing to a change in the temperature of the water during treatment the results expressed in per cent of saturation may show an increase in oxygen while there is in reality a marked decrease. For the oxygen consumed determination we used the Kübel method, boiling for two minutes.

In the bacteriological work we have followed the methods proposed by the Committee on Standard Methods of the American Public Health Association. The anaërobic counts were made in the earlier part of the work after the method of Wright (1901), later by a method proposed by Rickards (1904). Both methods are the same in principle, the oxygen being absorbed by pyrogallate of potassium.

#### GENERAL RESULTS OF THE EXPERIMENTS.

The analyses reported in Tables 5-10 represent six sets, each of 24 hourly samples. The first two series, of July and August, 1903, were intended to be typical of summer conditions. During the July run heavy rain fell while the August run was in fair weather. The third series in November represents late autumn conditions, and the fourth and fifth in December and

DIAGRAM I.

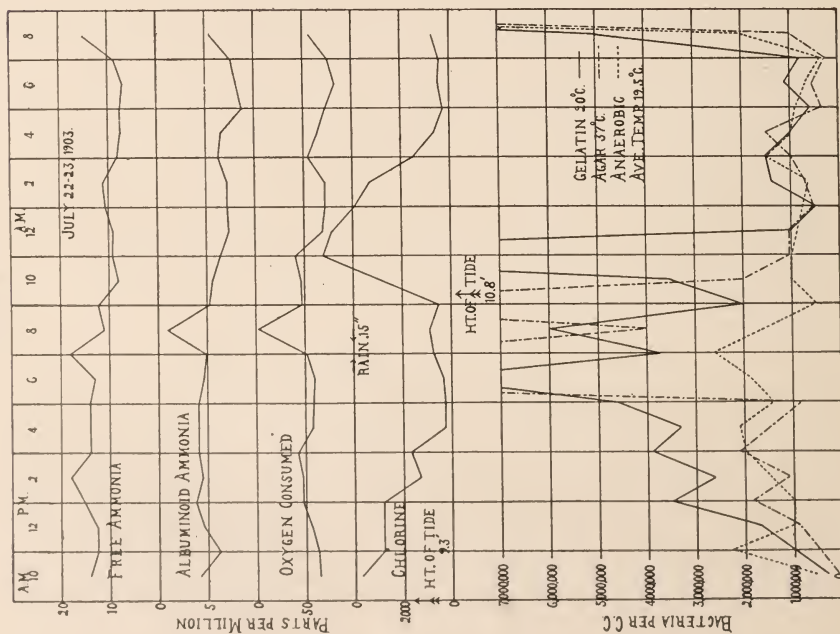


DIAGRAM I.

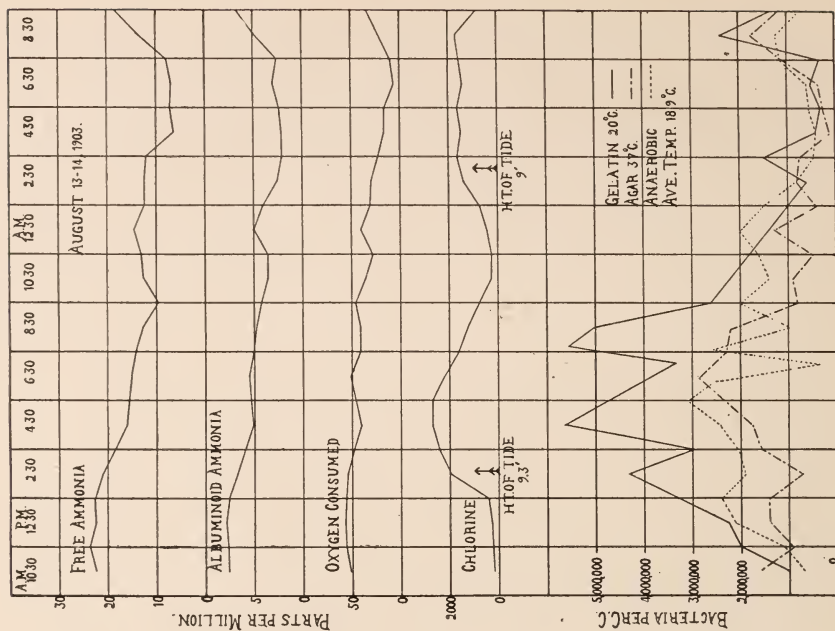


DIAGRAM III.

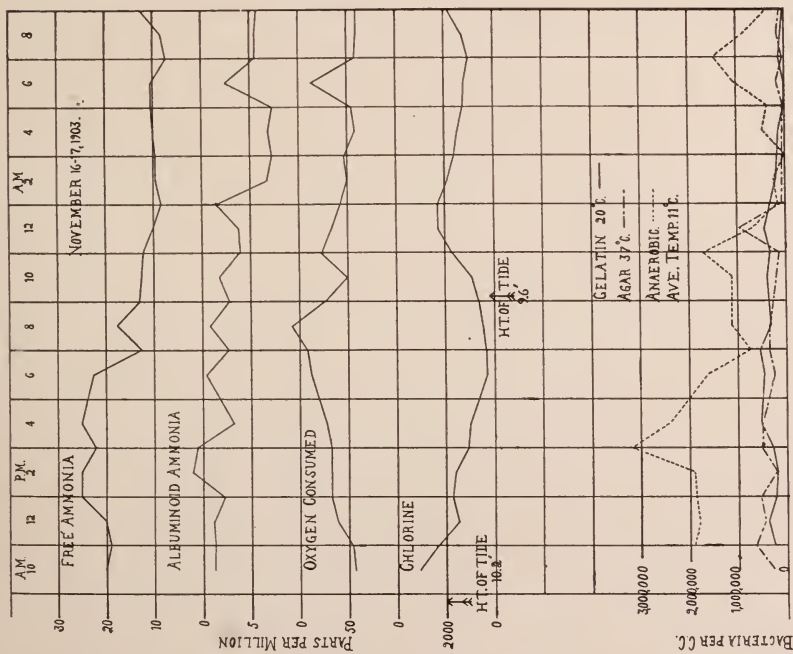


DIAGRAM IV.

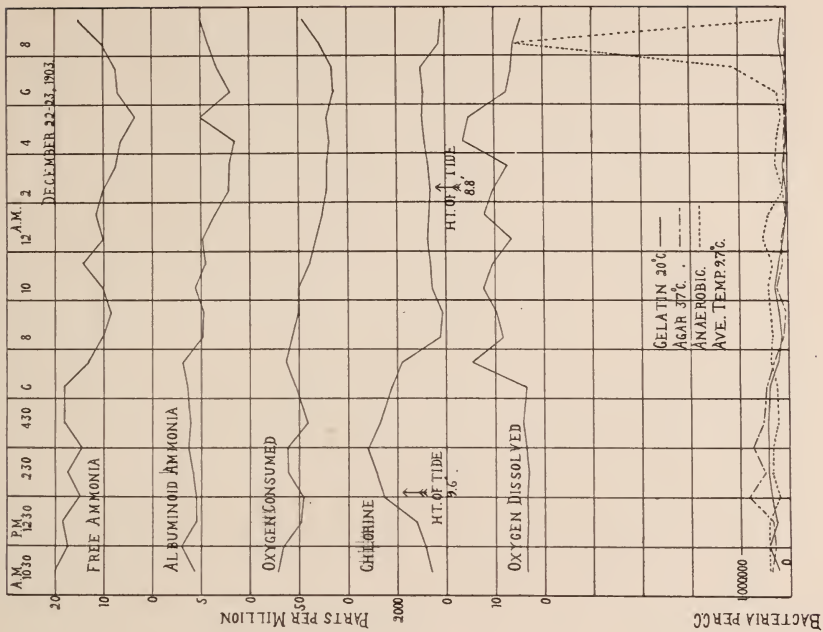


DIAGRAM V.

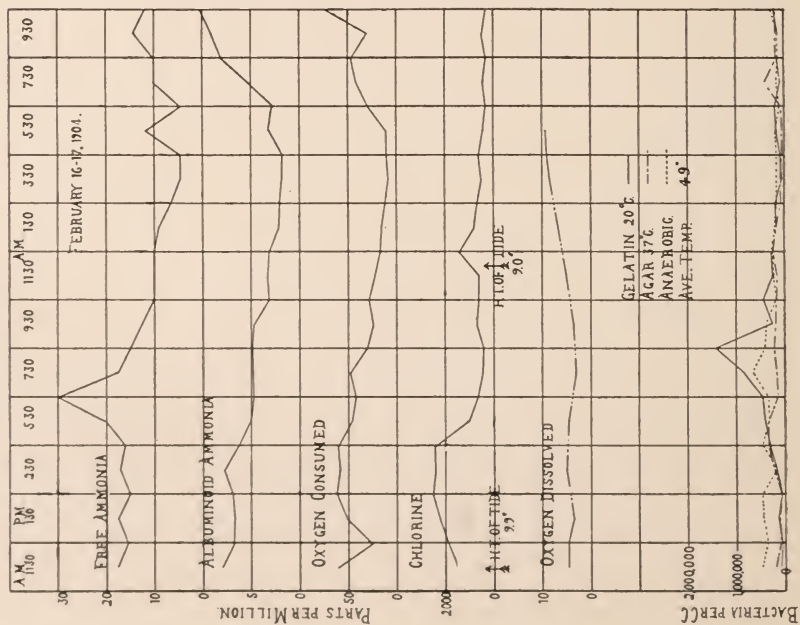


DIAGRAM VI.

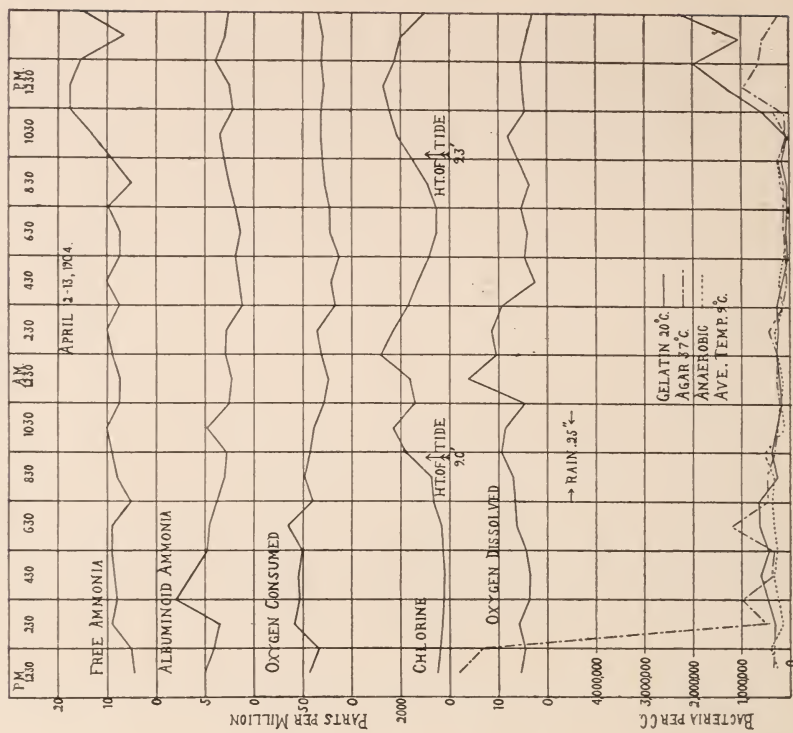




TABLE 5.

Parts per Million.

No.		NITROGEN AS					OXYGEN CONSUMED	OXYGEN DISSOLVED	CHLORINE	NUMBER OF BACTERIA IN ONE CUBIC CENTIMETER						
		Free Ammonia	Albuminoid Ammonia			Nitrates				Lactose Gelatin at 20°				Lactose Agar at 37°		Anaerobic Lactose Agar at 20°
			Total	Solution	Suspension					Nitrates	Acid	Liquefiers	Total	Acid	Total	
33	July 22 10 A. M. 11 12 1 P. M. 2 3 4 5 6 7 8 9	14.0	5.7	3.8	1.9	0.08	0.62	36	3,700	50,000	—	300,000	—	100,000	500,000	
34		12.5	3.7	3.5	0.2	0.08	—	38	2,800	50,000	—	1,000,000	100,000	500,000	2,300,000	
35		12.5	5.3	2.8	2.5	0.10	—	44	2,800	250,000	200,000	1,700,000	350,000	950,000	1,000,000	
36		15.0	6.1	2.7	3.4	0.08	0.83	52	2,800	850,000	150,000	3,500,000	1,100,000	1,850,000	1,000,000	
37		18.0	5.5	3.4	2.1	0.11	—	52	1,300	550,000	150,000	2,600,000	500,000	1,050,000	1,500,000	
38		14.0	5.8	3.4	2.4	0.10	—	57	1,700	400,000	200,000	3,900,000	650,000	2,100,000	2,000,000	
39		14.0	5.8	2.0	3.8	0.10	1.03	42	260	300,000	—	3,300,000	100,000	12,000,000	2,100,000	
40		14.0	5.8	2.3	3.5	0.14	—	41	260	550,000	150,000	4,600,000	300,000	800,000	1,400,000	
41		13.0	5.2	2.8	2.4	0.16	—	40	360	1,000,000	150,000	8,800,000	100,000	17,000,000	1,900,000	
42		18.0	5.0	2.8	2.2	0.17	0.53	47	678	400,000	—	3,700,000	1,300,000	9,200,000	2,600,000	
43		11.0	9.0	2.2	6.8	0.10	—	98	871	2,000,000	1,000,000	6,000,000	1,500,000	4,000,000	500,000	
44		13.0	4.7	1.9	2.8	0.07	—	52	543	—	—	2,000,000	2,500,000	12,500,000	1,000,000	
45	8.0	4.4	2.2	2.2	0.08	0.48	53	2,840	—	—	3,500,000	500,000	2,000,000	1,000,000		
46	9.0	3.5	1.2	2.3	0.13	—	58	5,100	10,000,000	1,000,000	15,000,000	500,000	1,000,000	1,000,000		
47	9.0	2.6	1.4	1.2	0.12	—	31	4,890	—	—	1,000,000	—	1,000,000	—		
48	July 23 1 A. M. 2 3 4 5 6 7 8 9	10.5	2.8	1.4	1.4	0.13	0.78	28	3,990	—	—	500,000	—	500,000	—	
49		11.0	2.8	1.3	1.5	0.05	—	26	3,330	200,000	200,000	1,400,000	200,000	650,000	650,000	
50		8.0	3.6	0.8	2.8	0.15	—	45	1,500	—	—	1,500,000	—	1,000,000	1,500,000	
51		7.2	3.4	0.9	2.5	0.15	0.53	35	632	—	—	1,000,000	—	1,500,000	1,000,000	
52		7.2	1.3	0.8	0.5	0.17	—	18	280	200,000	100,000	600,000	150,000	350,000	900,000	
53		7.0	1.9	1.1	0.8	0.15	—	26	440	200,000	150,000	1,100,000	150,000	550,000	700,000	
54		8.8	2.4	1.6	0.8	0.20	2.03	24	400	200,000	50,000	800,000	150,000	300,000	400,000	
55		15.0	4.5	2.4	2.1	0.10	—	44	720	400,000	300,000	5,000,000	400,000	1,000,000	2,000,000	
56		—	—	—	—	—	2.53	—	—	5,900,000	2,000,000	40,250,000	2,400,000	17,700,000	16,300,000	
Averages.....		11.7	4.3	2.1	2.2	0.11	1.09	43	1,805	1,400,000	414,000	4,710,000	682,000	3,733,000	2,010,000	
Weighted averages		11.8	4.4	2.1	2.3	0.12	0.98	45	2,100	1,370,000	428,900	4,620,000	701,500	3,000,000	1,978,000	

TABLE 6.  
Parts per Million.

No.	TIME	NITROGEN AS					OXYGEN CONSUMED	OXYGEN DISSOLVED	CHLORINE	NUMBER OF BACTERIA IN ONE CUBIC CENTIMETER							
		Free Ammonia	Albuminoid Ammonia			Nitrates				Nitrates	Lactose Gelatin at 20°			Lactose Agar at 37°		Anaerobic Lactose Agar at 20	
			Total	Solution	Suspension						Acid	Liquefiers	Total	Acid	Total		
August 13 A.M.																	
128	9:30-10:30	22.5	7.7	4.8	2.9	0.16	0.63	52	—	234	200,000	200,000	1,000,000	600,000	1,600,000	700,000	
129	10:30-11:30	24.0	7.7	4.7	3.0	0.14	—	57	—	236	700,000	100,000	2,000,000	450,000	900,000	1,150,000	
130	11:30-12:30	22.5	7.8	4.7	3.1	0.14	—	57	—	280	700,000	100,000	2,300,000	900,000	1,400,000	2,150,000	
P.M.																	
131	12:30-1:30	22.5	7.7	4.4	3.3	0.14	—	56	—	420	1,200,000	400,000	3,300,000	300,000	1,400,000	2,400,000	
132	1:30-2:30	21.0	6.7	3.2	3.5	0.06	0.45	55	—	1,920	1,000,000	350,000	4,350,000	600,000	700,000	1,800,000	
133	2:30-3:30	18.0	5.7	2.8	2.9	0.08	—	48	—	2,430	1,050,000	50,000	2,900,000	450,000	1,600,000	2,050,000	
134	3:30-4:30	16.0	5.0	2.7	2.3	0.10	—	41	—	2,550	2,900,000	500,000	5,700,000	600,000	1,750,000	2,450,000	
135	5:30-6:15	15.0	5.4	2.7	2.7	0.10	—	51	—	2,160	1,150,000	300,000	4,100,000	1,150,000	2,900,000	2,500,000	
136	6:15-7:00	14.5	5.1	2.8	2.3	0.10	0.43	48	—	1,830	1,500,000	300,000	3,200,000	600,000	1,900,000	800,000	
137	7:00-7:45	14.0	5.0	2.2	2.8	0.06	—	43	—	1,560	—	—	5,600,000	1,200,000	2,350,000	2,600,000	
138	7:45-8:30	13.0	4.9	3.1	1.8	0.06	—	42	—	1,200	1,150,000	250,000	5,020,000	1,600,000	2,200,000	900,000	
139	8:30-9:30	9.5	4.3	4	1.9	0.10	—	46	—	760	750,000	250,000	2,650,000	400,000	750,000	2,000,000	
140	9:30-10:30	12.5	3.4	5	0.9	0.12	0.55	34	—	220	—	—	—	300,000	950,000	1,400,000	
141	10:30-11:30	13.0	3.5	2.0	1.5	0.08	—	27	—	220	—	100,000	1,800,000	200,000	450,000	1,600,000	
142	11:30-12:30	14.5	4.9	1.9	3.0	0.08	—	40	—	390	—	—	—	700,000	1,300,000	2,000,000	
August 14 A.M.																	
143	12:30-1:30	12.5	3.7	1.9	1.8	0.10	—	28	—	760	—	—	—	250,000	350,000	1,500,000	
144	1:30-2:30	12.5	2.4	0.9	1.5	0.12	0.40	29	—	1,420	50,000	—	600,000	100,000	700,000	800,000	
145	2:30-3:30	12.0	2.0	1.5	0.5	0.14	—	21	—	1,600	—	—	1,500,000	200,000	700,000	450,000	
146	3:30-4:30	6.5	2.0	1.4	0.6	0.14	—	17	—	1,520	—	—	400,000	—	100,000	400,000	
147	4:30-5:30	7.0	2.2	1.5	0.7	0.16	—	16	—	1,540	—	—	300,000	200,000	300,000	550,000	
148	5:30-6:30	7.0	3.1	2.4	0.7	0.12	0.75	8	—	1,480	50,000	50,000	500,000	350,000	400,000	600,000	
149	6:30-7:30	8.0	2.5	2.0	0.5	0.14	—	15	—	1,560	—	—	300,000	200,000	1,100,000	1,100,000	
150	7:30-8:30	14.0	5.2	2.6	2.6	0.14	—	22	—	1,670	300,000	100,000	2,400,000	200,000	1,750,000	1,250,000	
151	8:30-9:30	18.5	6.7	3.9	2.8	0.16	—	34	—	954	400,000	400,000	1,850,000	450,000	1,500,000	700,000	
Averages . . . . .																	
		14.6	4.8	2.7	2.1	0.11	0.54	37	..	1,206	873,000	223,000	2,465,000	519,000	1,195,000	1,410,000	
Weighted averages		14.7	4.8	2.7	2.1	0.11	0.64	37	..	1,213	860,000	232,000	2,480,000	520,000	1,220,000	1,415,000	

TABLE 7.

Parts per Million

No.	TIME	NITROGEN AS					OXYGEN CONSUMED	OXYGEN DISSOLVED	CHLORINE	Lactose Gelatin at 20°				Lactose Agar at 37°		Anaerobic Lactose Agar at 20°
		Free Ammonia	ALBUMINOID AMMONIA			Nitrates				Nitrates	Acid	Liquefiers	Total	Acid	Total	
			Total	Solution	Suspension											
November 16																
428	9:00-10:00	20.0	8.8	3.4	5.4	0.14	—	2.30	3,050	—	—	—	270,000	—	270,000	—
	10:00-11:00	19.0	8.5	4.0	4.5	0.80	—	1.50	2,400	50,000	0	280,000	690,000	0	690,000	1,910,000
	11:00-12:00	20.0	8.8	4.2	4.6	8.80	—	1.40	1,560	60,000	0	400,000	400,000	0	440,000	1,810,000
429	12:00-1:00	25.0	7.7	4.8	2.9	0.90	—	1.10	1,750	30,000	0	230,000	500,000	0	560,000	1,870,000
	1:00-2:00	25.0	11.0	4.2	6.8	0.85	—	1.30	1,670	20,000	0	210,000	230,000	0	250,000	1,910,000
	2:00-3:00	22.0	10.5	4.0	6.5	0.68	—	0.70	1,100	60,000	0	300,000	520,000	0	550,000	3,160,000
	3:00-4:00	25.0	6.7	4.8	1.9	0.10	—	1.00	1,000	100,000	0	550,000	460,000	0	500,000	2,410,000
430	4:30-5:50	22.5	9.5	4.2	5.3	0.80	—	1.30	340	20,000	0	470,000	230,000	0	260,000	1,630,000
	7:00	12.5	7.3	3.4	3.9	0.00	—	—	360	70,000	0	540,000	320,000	0	360,000	750,000
	7:00-8:00	17.5	9.3	2.4	6.9	0.02	—	—	440	50,000	0	310,000	280,000	0	310,000	1,120,000
	8:00-9:00	13.0	7.3	2.4	4.9	0.00	—	—	600	40,000	0	360,000	260,000	0	280,000	1,120,000
431	9:00-10:00	12.5	8.3	2.8	5.5	0.01	—	—	900	40,000	0	390,000	210,000	0	210,000	1,120,000
	10:00-11:00	12.0	6.0	2.3	3.7	0.00	—	—	1,780	30,000	0	330,000	140,000	0	160,000	1,710,000
	11:00-12:00	10.0	6.1	1.9	4.2	0.00	—	—	2,300	80,000	0	450,000	960,000	0	960,000	690,000
November 17																
442	12:00-1:00	8.5	8.5	5.5	3.0	0.00	—	—	2,300	20,000	0	330,000	80,000	0	90,000	160,000
	1:00-2:00	9.3	3.3	1.3	2.0	0.00	—	—	1,900	20,000	0	190,000	60,000	0	60,000	250,000
	2:00-3:00	9.5	2.9	1.3	1.6	0.00	—	—	1,600	40,000	0	160,000	50,000	0	50,000	30,000
	3:00-4:00	10.0	3.0	1.3	1.7	0.00	—	—	1,520	20,000	0	130,000	50,000	0	60,000	460,000
443	4:00-5:00	10.5	2.6	.9	1.7	0.00	—	—	1,280	—	0	40,000	50,000	0	60,000	360,000
	5:00-6:00	10.5	7.6	1.5	6.1	0.00	—	—	1,100	—	0	40,000	140,000	0	190,000	1,070,000
	6:00-7:00	7.5	4.5	1.3	3.2	0.10	—	—	1,020	40,000	0	120,000	60,000	0	60,000	1,460,000
	7:00-8:00	8.5	4.5	1.9	2.6	0.60	—	—	1,290	—	0	80,000	160,000	0	170,000	830,000
444	8:00-9:00	12.5	4.3	2.0	2.3	0.80	—	—	1,860	30,000	0	40,000	90,000	0	100,000	340,000
Averages, . . . . .		14.9	6.8	3.0	3.8	0.28	—	1.32	1,436	43,000	0	258,000	270,000	0	288,000	1,188,000
Weighted averages		14.9	6.8	3.0	3.8	.29	—	1.32	1,439	43,000	0	272,000	271,000	0	288,000	1,193,000



TABLE 8.  
Parts per Million

No.	TIME	NITROGEN AS						OXYGEN CONSUMED	OXYGEN DISSOLVED	CHLORINE	NUMBER OF BACTERIA IN ONE CUBIC CENTIMETER					
		Free Am- monia	ALBUMINOID AMMONIA			Ni- trates	Ni- trites				Lactose Gelatin at 20°	Lactose Agar at 37°		Anaerobic Lactose- Agar at 20°		
			Total	Solu- tion	Sus- pen- sion							Acid	Total			
															Total	Acid
December 22																
	A. M.															
543	9:30-10:30	20.0	5.7	3.6	2.1	0	1.80	72	3.40	600	180,000	—	230,000	360,000	380,000	420,000
544	10:30-11:30	17.5	7.0	3.6	3.4	0	0.40	66	3.50	860	160,000	10,000	410,000	240,000	300,000	430,000
545	11:30-12:30	18.5	5.5	3.5	2.0	0	0.40	49	3.30	1,200	200,000	10,000	280,000	300,000	340,000	410,000
P. M.																
546	12:30-1:30	15.0	5.5	3.4	2.1	0	1.80	46	3.50	2,500	290,000	5,000	380,000	830,000	840,000	200,000
547	1:30-2:30	17.5	5.8	2.9	2.9	0	0.60	61	3.00	2,800	400,000	5,000	420,000	370,000	500,000	360,000
548	2:30-3:30	14.5	6.2	3.0	3.2	0	1.00	62	3.50	3,200	400,000	10,000	420,000	760,000	790,000	360,000
549	3:30-4:30	18.0	6.1	3.7	2.4	0	0.20	41	4.00	2,700	350,000	15,000	430,000	430,000	540,000	220,000
550	5:00-6:00	18.0	6.3	2.9	2.4	0	0.20	53	3.30	2,200	310,000	—	410,000	320,000	230,000	230,000
551	6:00-7:00	13.0	6.9	4.0	2.9	0.05	0.20	63	14.70	1,790	180,000	—	210,000	280,000	310,000	370,000
552	7:00-8:00	10.0	4.9	2.9	2.0	0.05	0.20	57	8.40	240	130,000	—	140,000	70,000	100,000	320,000
553	8:00-9:00	8.5	4.7	2.2	2.5	0.10	0.20	50	9.70	190	190,000	5,000	190,000	50,000	60,000	370,000
554	9:00-10:00	10.0	5.5	2.3	2.2	0	0.10	50	12.10	490	260,000	10,000	280,000	210,000	250,000	400,000
555	10:00-11:00	14.0	4.5	2.4	2.1	0	0.10	39	10.30	600	200,000	—	200,000	100,000	120,000	330,000
556	11:00-12:00	10.0	4.7	2.6	2.1	0.05	1.00	—	6.50	710	100,000	—	110,000	110,000	130,000	520,000
December 23																
	A. M.															
557	12:00-1:00	11.5	3.5	1.7	1.8	0.20	0.10	26	12.00	660	40,000	—	50,000	5,000	50,000	410,000
558	1:00-2:00	10.0	2.2	1.6	0.6	0.05	0.10	22	10.60	640	50,000	—	70,000	100,000	100,000	100,000
559	2:00-3:00	7.5	2.1	1.0	1.1	0	0.10	21	7.40	670	100,000	5,000	100,000	70,000	80,000	270,000
560	3:00-4:00	6.5	1.5	0.9	0.6	0	0.20	19	16.50	760	20,000	—	30,000	70,000	70,000	220,000
561	4:00-5:00	3.5	5.0	4.2	0.8	0	0.20	21	15.10	980	40,000	—	40,000	40,000	40,000	130,000
562	5:00-6:00	7.0	2.0	0.8	1.2	0	0.30	14	7.80	900	10,000	—	10,000	50,000	210,000	210,000
563	6:00-7:00	7.5	3.3	1.6	1.7	0	0.20	16	6.50	1,000	30,000	—	40,000	5,000	20,000	1,860,000
564	7:00-8:00	10.1	4.2	2.9	1.3	0	0.20	28	6.00	260	160,000	—	160,000	—	10,000	5,550,000
565	8:00-9:00	15.0	5.0	2.9	2.1	0	0.20	45	4.60	180	80,000	—	100,000	20,000	50,000	260,000
Averages.....												8,000	205,000	218,000	242,000	606,000
Weighted averages												7,100	200,000	210,000	234,000	625,000



TABLE 9.

Parts per Million

No.	Time	NITROGEN AS										NUMBER OF BACTERIA IN ONE CUBIC CENTIMETER				
		Free Ammonia	Albuminoid Ammonia		Nitrates	Nitrates	OXYGEN CONSUMED	OXYGEN DISSOLVED	Lactose Gelatin at 29°				Lactose Agar at 37°		Anaerobic Lactose Agar at 29°	
			Total	Suspension					Acid	Liquefiers	Total	Acid	Total			
610	February 16	17.5	8.0	3.5	4.5	—	61	4.80	1,500	55,000	0	60,000	100,000	180,000	475,000	
611	11:30 A. M.	15.5	6.9	3.0	3.9	—	25	4.90	1,900	5,000	0	45,000	55,000	90,000	365,000	
612	12:30 P. M.	17.5	6.6	3.4	3.2	—	52	3.60	2,200	10,000	10,000	135,000	55,000	110,000	430,000	
613	2:30	15.0	6.7	3.2	3.5	—	61	—	2,500	30,000	10,000	80,000	50,000	95,000	485,000	
614	3:30	17.0	7.7	3.8	3.9	—	57	5.00	2,400	40,000	5,000	185,000	130,000	290,000	195,000	
615	4:30	16.0	6.3	3.6	2.7	—	60	—	2,400	115,000	10,000	300,000	170,000	335,000	475,000	
616	5:30	20.0	5.0	3.6	1.4	—	46	4.70	1,000	110,000	5,000	400,000	185,000	310,000	305,000	
617	6:30	30.0	4.7	2.7	2.0	—	42	—	540	330,000	5,000	430,000	70,000	145,000	325,000	
618	7:30	17.5	4.7	3.0	1.7	—	48	3.00	440	590,000	45,000	820,000	100,000	155,000	650,000	
619	8:30	15.0	4.9	2.6	2.3	—	30	—	400	340,000	35,000	1,400,000	185,000	200,000	400,000	
620	9:30	12.5	4.6	2.4	2.2	—	24	4.20	700	85,000	0	245,000	130,000	190,000	380,000	
621	10:30	10.0	3.1	1.6	1.5	—	27	—	600	315,000	10,000	420,000	100,000	175,000	180,000	
622	11:30	10.0	3.5	2.1	1.4	—	22	5.00	600	120,000	5,000	235,000	125,000	165,000	215,000	
623	February 17	10.0	3.2	1.6	1.6	—	16	—	1,400	180,000	30,000	230,000	115,000	215,000	225,000	
624	12:30 A. M.	9.0	2.3	1.0	1.3	—	15	6.90	800	115,000	5,000	135,000	70,000	145,000	185,000	
625	1:30	6.5	2.2	1.1	1.1	—	11	—	700	60,000	15,000	190,000	20,000	65,000	145,000	
626	2:30	3.80	4.5	1.8	1.1	0.01	9	8.20	500	0	0	20,000	35,000	45,000	130,000	
627	3:30	4.5	1.8	0.9	0.9	—	10	9.00	600	20,000	5,000	60,000	15,000	25,000	150,000	
628	4:30	11.5	3.1	1.9	1.2	—	11	9.30	440	90,000	0	130,000	10,000	30,000	195,000	
629	5:30	4.5	2.7	2.0	0.7	—	28	—	300	65,000	5,000	170,000	35,000	65,000	100,000	
630	6:30	10.0	5.3	3.7	1.6	—	41	—	400	40,000	0	85,000	135,000	400,000	145,000	
631	7:30	10.0	8.0	6.0	2.0	—	46	—	280	50,000	10,000	115,000	95,000	135,000	115,000	
632	8:30	14.0	9.0	6.2	2.8	—	31	—	480	70,000	0	115,000	110,000	145,000	115,000	
633	9:30	11.5	5.2	3.0	2.2	—	74	—	300	110,000	25,000	200,000	145,000	190,000	290,000	
633	10:30	11.5	5.2	3.0	2.2	—	74	—	300	110,000	25,000	200,000	145,000	190,000	290,000	
Averages, .....		12.9	4.9	2.8	2.1	0.01	35	5.72	974	122,700	9,800	258,400	93,300	158,750	278,500	
Weighted averages		12.8	4.9	2.8	2.1	0.01	35	5.73	951	122,000	8,800	261,000	93,500	156,000	294,000	

TABLE 10.

Parts per Million

No	TIME	NITROGEN AS					OXYGEN CONSUMED	OXYGEN DISSOLVED	CHLORINE	NUMBER OF BACTERIA IN ONE CUBIC CENTIMETER					
		Free Am- monia	Albuminoid Ammonia			Ni- trates				Ni- trates	Lactose Gelatin at 20°			Lactose Agar at 37°	
			Total	Solu- tion	Sub- pen- sion						Acid	Liquefiers	Total	Acid	Total
673	April 12 12:30 P. M.	4.5	5.0	2.2	2.8	—	44	5.6	500	158,000	75,000	350,000	6,500,000	230,000	
674	1:30	5.0	4.2	2.6	1.6	—	34	4.8	400	—	—	—	6,000,000	400,000	
675	2:30	9.0	3.5	2.6	0.9	—	59	5.7	300	60,000	40,000	300,000	140,000	150,060	
676	3:30	8.5	8.0	3.4	4.6	0.01	53	3.9	300	—	—	—	930,000	230,000	
677	4:30	—	—	—	—	0.01	54	3.4	200	60,000	30,000	700,000	350,000	315,000	
678	5:30	9.0	4.7	2.8	1.9	0.00	51	4.4	—	160,000	40,000	415,000	270,000	280,000	
679	6:30	9.0	4.5	2.4	2.1	0.00	66	6.1	390	105,000	65,000	615,000	1,200,000	340,000	
680	7:30	5.0	3.7	2.3	1.4	—	40	6.4	620	200,000	30,000	630,000	150,000	380,000	
681	8:30	8.0	3.3	2.0	1.3	—	47	6.9	770	85,000	10,000	260,000	450,000	305,000	
682	9:30	9.0	2.7	2.1	0.6	—	42	9.1	1,810	65,000	0	380,000	300,000	420,000	
683	10:30	10.0	4.7	1.6	3.1	—	39	8.5	2,390	90,000	20,000	260,000	200,000	120,000	
684	11:30	7.5	2.5	1.6	0.9	—	28	4.9	1,490	30,000	0	150,000	160,000	190,000	
685	April 13 12:30 A. M.	7.5	2.2	1.4	0.8	—	24	16.1	1,600	50,000	0	200,000	210,000	160,000	
686	1:30	9.0	2.7	1.6	1.1	—	31	10.1	2,820	55,000	25,000	250,000	200,000	235,000	
687	2:30	10.0	2.7	1.4	1.3	—	34	11.3	2,310	85,000	20,000	230,000	390,000	315,000	
688	3:30	7.5	1.1	0.8	0.3	—	17	9.9	1,740	55,000	0	255,000	140,300	160,000	
689	4:30	10.0	1.5	1.0	0.5	—	23	2.3	1,280	30,000	5,000	150,000	35,000	230,000	
690	5:30	7.5	1.9	0.8	1.1	—	14	4.5	800	10,000	15,000	65,000	80,000	120,000	
691	6:30	7.5	1.4	1.3	0.1	—	23	4.2	500	5,000	25,000	100,000	90,000	110,000	
692	7:30	9.5	1.8	1.5	0.3	—	22	5.9	540	10,000	0	95,000	110,000	70,000	
693	8:30	5.0	2.5	1.6	0.9	0.00	27	3.7	920	35,000	0	55,000	120,000	175,000	
694	9:10	10.0	3.7	2.8	0.9	0.00	39	8.9	1,560	130,000	10,000	195,000	230,000	250,000	
695	10:30	13.0	3.5	2.2	1.3	—	31	8.8	2,100	0	0	55,000	90,000	30,000	
696	11:30	17.5	2.2	2.0	0.2	—	32	4.5	2,400	320,000	15,000	550,000	150,000	160,000	
697	12:30 P. M.	17.5	2.5	2.2	0.3	0.00	29	—	2,700	900,000	30,000	1,300,000	930,000	980,000	
698	1:30	15.4	4.0	2.4	1.6	—	31	5.3	2,200	750,000	150,000	2,000,000	580,000	680,000	
699	2:30	6.5	3.0	2.2	0.8	—	30	4.3	2,000	—	—	—	—	—	
700	3:30	15.0	2.7	2.0	0.7	—	32	3.0	1,000	650,000	5,000	1,080,000	550,000	—	
Average for 24 hrs.		8.6	3.2	1.9	1.3	0.003	36	6.6	1,205	85,600	28,300	283,600	799,800	231,300	
Average for 28 hrs.		9.0	3.2	2.0	1.2	0.003	36	5.3	1,320	219,000	32,600	497,000	741,600	231,300	
Weighted average, 28 hrs. ....		9.0	3.2	2.0	1.2	0.003	36	5.3	1,320	219,000	32,600	477,000	741,600	231,310	

February, are typical of winter weather. Considerable snow and rain fell during the November run. Finally the last series (in April), followed heavy rain and spring thaws.

The analyses recorded in detail in Tables 5-10 are plotted in graphic form in Diagrams I-VI. Inspection of the curves shows, first, the general relation of the day and night flow. The nitrogenous bodies, indicated by the ammonias, and the carbonaceous constituents as measured by the oxygen consumed, rise to a maximum between 11:30 A. M. and 2:30 P. M., and fall to a minimum between 3 and 6 A. M. The curves for the dissolved oxygen naturally exhibit a reciprocal relation the amount increasing regularly from early evening to 6 or 7 A. M. The curves are most even and show the widest diurnal variation in the winter runs when the surface water was largely excluded and in the August series during the dry summer period.

Bacteriological analyses of sewage have never been carried out with so much detail so far as we are aware, and they bring out several points of interest. First, the enormous diurnal variations in warm weather are strikingly manifest in the diagrams of the July and August tests. The maximum occurs not at 11:30 A. M. when the organic constituents are highest, but from four to six hours later, at 4:30 P. M. We are inclined to attribute this mainly to the fact that the large amount of organic matter which passes in the morning is from the adjacent regions of Boston proper, and is comparatively fresh and undecomposed, while that arriving in the afternoon comes from the upper part of the system, so that the bacteria present have had time to multiply. The fact that the other four curves indicate no such enormous increase shows the important effect of the seasonal variations of temperature upon the multiplication of bacteria. The diurnal variation is, however, clearly shown even in December and February, although the absolute numbers are so small that the differences are somewhat obscured by the scale of our diagrams.

The effects of dilution with rainwater are shown with great clearness by the curves for the April analyses. The actual amount of nitrogenous matter present was much lower than at any other time, while the values for oxygen consumed remained as



high as in August and February, and not far below the July and December figures. This is attributable to the fact that while street washings are poor in nitrogen as compared with domestic sewage, they contain considerable carbonaceous matter derived from horse droppings, etc. The normal diurnal variations are, of course, somewhat obscured in the presence of much surface water, and this phenomenon is also evident in the April analyses.

#### GENERAL DIURNAL VARIATIONS IN THE COMPOSITION OF BOSTON SEWAGE.

Fuller (1903) and others have emphasized the importance of the hourly variations in the composition of sewage, and Goodnough and Johnson (1899) have published numerous series of analyses which bring out with clearness the extent of the variations. The absurdity of calculating sewage purification on the basis of analyses made during parts of the day when the sewage has twice its normal strength must be obvious, and yet this solecism is still commonly committed. Not only the hourly analyses but the hourly variations in flow should be considered; and in each of our tables there is given first the crude average for each constituent, and second, the true average corrected by weighting each analysis according to the amount of sewage flowing. In our case the correct average differs but little from the crude average, since the Moon Island sewer is usually full and shows but little variation in flow. Under other conditions, however, this allowance for hourly variations in flow would become of great importance.

We have calculated for the principal features of the analyses in Tables 5-10 the ratios which each hourly figure bear to the general average for the day, and have averaged the results obtained for each hour in the six different tests. The final figures in Table 11 probably form a fairly correct measure of the hourly variations in Boston sewage, and bring out some points not made clear in the diagrams. The maximum of albuminoid ammonia and oxygen consumed is seen to fall between 10 A. M. and 2 P. M., while the free ammonia is highest between 1 P. M. and 8 P. M. The latter thus follows the bacteria which is natural, since in all water and sewage analyses free ammonia, the product of bacterial decom-



position, and the bacteria which form it, are found to vary together. The dissolved oxygen is highest at 4 to 5 A. M., when ammonia, oxygen consumed, and bacteria alike reach their minimum. For purposes of comparison we have calculated similar

TABLE 11.

RATIO OF ANALYSES FOR EACH HOUR TO THE AVERAGE ANALYSIS OF THE TWENTY-FOUR HOURS (AVERAGE OF SIX SERIES).

TIME	FREE AMMONIA	ALBUMINOID AMMONIA		OXYGEN CONS.	OXYGEN DISS.	CHLORINE	BACTERIA		
		Total	Susp.				Gela- tin, 20°	Agar, 37°	Ana- erobic
10 A. M. ....	1.29	1.33	1.35	1.31	1.26	1.15	.77	.89	.60
11 " .....	1.27	1.27	1.19	1.19	.84	1.12	.81	.98	1.13
12 Noon .....	1.25	1.24	1.21	.98	1.35	1.17	.85	.70	1.04
1 P. M. ....	1.35	1.25	1.21	1.20	.96	1.61	1.04	1.17	1.11
2 " .....	1.40	1.29	1.49	1.31	1.17	1.71	1.05	.81	1.22
3 " .....	1.21	1.22	1.29	1.21	.94	1.71	1.14	1.42	1.16
4 " .....	1.31	1.07	1.03	1.09	.64	1.66	1.49	1.80	1.31
5 " .....	1.16	1.02	1.11	1.00	.84	1.18	1.09	1.15	1.02
6 " .....	1.38	1.07	.97	1.12	.74	1.06	1.43	1.64	.82
7 " .....	1.14	1.04	.96	1.20	1.18	.71	1.30	1.35	1.16
8 " .....	.92	1.20	1.42	1.00	.90	.52	1.90	.97	.86
9 " .....	.81	1.02	1.05	1.09	1.21	.58	.91	1.11	.95
10 " .....	.91	.97	.93	.96	1.13	.95	1.08	.72	.64
11 " .....	.97	.80	.75	.96	.80	1.30	1.67	.77	.98
12 Midnight....	.75	.95	.94	.90	.74	1.33	1.10	2.25	.93
1 A. M. ....	.76	.86	.82	.71	1.07	1.04	.55	1.22	.85
2 " .....	.80	.61	.53	.74	1.21	.78	.48	.38	.40
3 " .....	.68	.83	1.06	.72	1.11	.63	.40	.47	.49
4 " .....	.52	.46	.51	.69	1.64	.57	.73	.30	.57
5 " .....	.75	.64	.56	.66	1.42	.90	.57	.21	.55
6 " .....	.62	.76	.79	.82	1.13	.40	.65	.59	.64
7 " .....	.63	.77	.67	.71	1.11	.47	.77	.67	1.24
8 " .....	.87	1.06	.91	.92	1.15	.56	.61	.63	.73
9 " .....	.95	1.15	.94	.93	1.04	1.04	2.18	1.31	.59

ratios for the six 24-hour runs reported by Goodnough and Johnson (1899), as Fuller (1903) has done for one of them. The results are presented in Table 12. Representing only a single series, the variations are less even than in our own table; yet they suggest distinct differences between the sewages studied. Marlborough, Spencer, and Natick sewages appear to be most concentrated in the afternoon, which is probably due to the length of the sewers above the point at which samples were taken.

TABLE 12.

RATIO OF ANALYSES AT DIFFERENT HOURS TO THE AVERAGE ANALYSIS OF THE  
TWENTY-FOUR HOURS, IN SIX MASSACHUSETTS COMMUNITIES.

Spencer—June, 1898.

Hour	Rate of Flow	Residue on Evaporation			Nitrogen as		Oxygen Consumed	Chlorine
	Gals. per 24 hrs.	Total	Dissolved	Suspended	Free Ammonia	Albuminoid Ammonia		
8-9 A.M. . .	1.13	1.18	1.02	1.68	2.25	1.94	1.44	.90
10-11 " . .	.98	1.77	1.30	3.20	2.20	1.58	1.58	1.35
12-1 P.M. . .	1.29	1.11	1.03	1.36	1.85	1.49	1.35	.96
2-3 " . .	1.20	1.47	1.27	2.07	1.76	1.49	1.90	1.06
4-5 " . .	1.18	1.16	1.54	0.00	1.76	1.17	1.24	2.33
6-7 " . .	1.06	.85	.94	.58	1.68	.66	.76	.96
8-9 " . .	.97	.97	.96	.98	1.63	1.12	.96	.88
10-11 " . .	.90	.83	.81	.89	.97	.66	.84	.65
12-1 A.M. . .	.90	.53	.65	.15	.53	.33	.27	.59
2-3 " . .	.80	.52	.68	.05	.21	.15	.21	.56
4-5 " . .	.76	.48	.63	.03	.12	.14	.16	.55
6-7 " . .	.97	.59	.73	.18	.87	.34	.39	.69

Brockton—Outlet of Main Sewer, June, 1898.

8-9 A.M. . .	...	.35	.53	1.10	1.80	1.63	.24	.42
10-11 " . .	...	1.35	1.09	1.74	1.62	2.07	1.74	.82
12-1 P.M. . .	...	1.20	1.30	1.07	1.70	1.54	1.60	1.24
2-3 " . .	...	1.30	1.37	1.21	1.66	1.44	1.34	1.45
4-5 " . .	...	1.51	1.45	1.61	1.40	1.44	1.41	1.72
6-7 " . .	...	1.02	1.13	.86	1.16	1.06	1.34	.89
8-9 " . .	...	.91	1.06	.70	1.32	1.10	1.03	1.01
10-11 " . .	...	1.15	1.40	.79	1.04	1.04	1.17	1.96
12-1 A.M. . .	...	1.17	.92	1.52	.81	1.08	.90	.85
2-3 " . .	...	1.31	.68	2.25	.59	.48	.68	.64
4-5 " . .	...	.38	.57	.94	.37	.30	.32	.55
6-7 " . .	...	.33	.52	.49	.24	.16	.25	.45

Framingham—Main Sewer, June, 1898.

9-10 A.M. . .	...	1.32	1.29	1.40	1.87	1.77	1.69	1.25
11-12 " . .	...	1.03	1.07	.94	1.45	1.26	1.16	1.04
1-2 P.M. . .	...	1.63	1.30	2.50	1.34	1.49	1.59	1.00
3-4 " . .	...	1.31	1.20	1.60	1.14	1.70	.23	1.24
5-6 " . .	...	1.04	.97	1.23	1.45	1.13	1.12	.76
7-8 " . .	...	1.13	1.03	1.40	1.48	1.19	1.18	.86
9-10 " . .	...	.82	.66	1.23	.67	.98	.82	.63
11-12 " . .	...	.57	.69	.28	.78	.43	.49	.77
1-2 A.M. . .	...	.47	.63	.05	.25	.26	.20	.60
3-4 " . .	...	.50	.67	.08	1.34	.21	.19	.58
5-6 " . .	...	.97	1.22	.34	.23	.34	.31	1.58
7-8 " . .	...	1.20	1.30	.98	1.22	1.23	1.00	1.46

TABLE 12.—Continued.

Marlborough — Main Sewer, June, 1898.

Hour	Rate of Flow	Residue on Evaporation			Nitrogen as		Oxygen Consumed	Chlorine
	Gals. per 24 hrs.	Total	Dissolved	Suspended	Free Ammonia	Albuminoid Ammonia		
9-10 A.M. . .	1.03	.76	.79	.68	1.08	.84	.58	.75
11-12 " . .	1.13	1.57	1.58	1.55	2.51	1.71	1.55	2.55
1- 2 P.M. . .	1.10	1.15	1.06	1.36	1.65	1.95	1.19	.96
3- 4 " . .	1.17	1.36	1.19	1.78	1.12	1.35	1.72	1.09
5- 6 " . .	1.27	1.46	1.14	2.30	1.19	1.69	1.96	.94
7- 8 " . .	1.05	.97	.99	.94	.93	1.01	1.26	.79
9-10 " . .	1.02	.99	1.09	.75	.86	.87	.91	1.28
11-12 " . .	.94	.86	.92	.72	.82	.75	.78	.82
1- 2 A.M. . .	.90	.70	.97	.48	.59	.48	.52	.67
3- 4 " . .	.80	.57	.74	.14	.26	.24	.29	.57
5- 6 " . .	.77	.54	.69	.16	.86	.10	.12	.52
7- 8 " . .	.85	.53	.73	.00	.76	.07	.12	.56

Natick — Main Sewer, June, 1898.

9-10 A.M. . .	...	.97	1.02	.40	2.35	1.47	.64	1.26
11-12 " . .	...	1.26	1.20	1.97	1.69	1.45	1.25	1.73
1- 2 P.M. . .	...	.99	1.02	.77	1.47	1.62	1.41	.94
3- 4 " . .	...	1.10	1.02	2.00	.70	.68	1.68	.96
5- 6 " . .	...	1.22	1.02	3.27	1.10	1.24	1.28	1.10
7- 8 " . .	...	1.18	1.04	2.75	1.33	1.73	2.23	.91
9-10 " . .	...	.99	1.06	.19	.90	.70	1.18	1.14
11-12 " . .	...	.85	.93	.05	.57	1.05	.59	.89
1- 2 A.M. . .	...	.87	.94	.14	.57	.51	.43	.79
3- 4 " . .	...	.86	.94	.10	.33	.23	.30	.77
5- 6 " . .	...	.84	.90	.29	.43	.75	.36	.75
7- 8 " . .	...	.86	.94	.05	.78	.70	.89	.79

Gardner — July, 1898.

9-10 A.M. . .	1.24	1.43	1.26	1.80	1.50	1.80	1.58	.99
11-12 " . .	1.16	1.60	1.59	1.62	1.57	1.55	1.67	1.14
1- 2 P.M. . .	1.23	1.29	1.31	1.25	1.07	1.07	1.10	1.27
3- 4 " . .	1.16	1.42	1.14	2.04	1.13	1.41	1.41	1.20
5- 6 " . .	1.07	1.23	1.37	.93	1.16	.99	.92	2.17
7- 8 " . .	1.03	.97	1.05	.79	1.41	1.17	1.10	1.16
9-10 " . .	.92	.67	.76	.49	.84	.66	.85	.75
11-12 " . .	.85	.84	.76	1.01	.98	.87	.99	.77
1- 2 A.M. . .	.74	.35	.43	.16	.35	.31	.22	.53
3- 4 " . .	.70	.28	.39	.06	.08	.15	.16	.38
5- 6 " . .	.77	.32	.44	.08	.16	.14	.19	.38
7- 8 " . .	1.14	.69	.74	.59	1.01	.87	.82	.61

By comparison with the tables above, it appears that the diurnal variations in Boston sewage are less wide than those characteristic of smaller towns. This is natural, since the long sewers of the larger system must tend to equalize local variations.

The practical conclusion of this work is, of course, the derivation of a constant by which the true composition of the sewage for 24 hours may be deduced from analyses made at a given time,—in the case of our experiments during the late morning. Reference to Table 11 shows that the ammonia and oxygen consumed ratios for Boston in the morning hours from 10 A. M. till noon range not far from 1.25 while the bacterial ratios on gelatin and agar are approximately .8. In using our routine daily analyses made on samples collected at this time as a measure of the average composition of the sewage this factor may be used as a correction.

#### MONTHLY VARIATIONS IN THE COMPOSITION OF BOSTON SEWAGE.

Our data for the study of seasonal variations consist of a series of daily analyses made during the last six months of 1903 and during April, May, and June, 1904. The examinations were unfortunately interrupted from January to March 1904. Averages of the analyses by months are shown in Table 13 with the pumpage at Moon Island in billion gallons per month as a correction factor. It must be noted that this is the total flow from the whole South Metropolitan system, not merely that which passes Albany Street; but the relative proportion of the latter must by months be practically constant. The crude average is first shown in the table, and next the corrected average, obtained by multiplying the analyses for each month by the flow for that month and dividing the sum by the total flow.

The monthly variations are better seen by a comparison of the ratios as they are shown in Table 14. The seasonal differences are much less important than those which subsist between the night and day flows; yet there is a well marked minimum in May, followed by a steady rise, culminating in November or December. The strength of the sewage bears a general inverse relation to the amount flowing, although from October to December both flows and analyses were high.



TABLE 13.

BOSTON SEWAGE—MONTHLY AVERAGE OF DAILY SAMPLES.

DATE	FLOW, BILLION GALLONS.	NITROGEN AS						OXYGEN CONSUMED, TOTAL
		Albuminoid NH <sub>3</sub>			Free NH <sub>3</sub>	Ni- trites	Ni- trates	
		Total	Solu- tion	Sus- pension				
April, 1904 .....	4.0	5.2	2.8	2.3	15.8	.09	.00	41
May, 1904 .....	4.2	5.7	2.5	3.2	14.9	.14	.00	31
June, 1904 .....	3.8	7.0	3.0	4.0	19.8	.22	.48	43
July, 1903 .....	3.4	6.3	3.2	3.0	18.2	.12	.48	47
August, 1903 .....	3.3	7.1	3.8	3.3	22.4	.12	.00	45
September, 1903 ....	3.4	6.3	3.0	3.2	21.4	.18	.00	45
October, 1903 .....	3.8	6.3	3.4	2.9	20.5	.80	...	51
November, 1903 .....	3.8	6.1	3.0	3.2	22.3	.20	...	53
December, 1903 .....	4.2	6.6	3.6	3.0	24.0	.42	...	50
Crude average ...	3.8	6.3	3.1	3.1	19.9	.26	.16	45
Average corrected for flow .....	...	6.2	3.1	3.1	19.9	.26	.15	45
Final ave. cor. for diurnal variation		5.0	2.5	2.5	15.9	.21	.12	36

In the last line of Table 13 we have applied the diurnal variation factor, dividing the corrected averages above by 1.25.

TABLE 14.

BOSTON SEWAGE—RATIO OF MONTHLY ANALYSES TO GENERAL AVERAGE.

MONTH	FLOW	ALBUMINOID AMMONIA			FREE AMMONIA	OXYGEN CONSUMED
		Total	Solution	Suspension		
April, 1904 .....	105	83	90	74	79	91
May, 1904 .....	115	91	80	103	74	69
June, 1904 .....	100	115	96	128	100	95
July, 1903 .....	89	99	104	97	91	104
August, 1903 .....	87	113	120	107	112	100
September, 1903 ...	89	99	97	104	107	100
October, 1903 .....	100	100	108	93	103	113
November, 1903 ....	100	97	95	101	112	118
December, 1903 ....	115	105	116	95	120	111
Nine months ....	100	100	100	100	100	100

For comparison we have calculated the monthly ratios for four other cities and towns, which are given in Table 15. The original analyses for Andover and Lawrence were obtained from the report of the Massachusetts State Board of Health for 1901

(Clark, 1902); the Worcester figures were taken from the reports of the superintendent of sewers from 1900 to 1903 (average of the four years), and the Brockton analyses from the reports of the City Engineer for 1900 and 1901 (average for the two years). All show October and November to be much higher than the other months. September is also high except at Andover; January and February are well above the average at Worcester; and January, February, and March at Lawrence. The minimum in each case occurs in April or May. There is, then, a yearly cycle in the composition of sewage as definite, if not as wide in range, as the diurnal cycle.

TABLE 15.

RATIOS OF THE MONTHLY AVERAGES OF SEWAGE ANALYSES TO THE YEARLY AVERAGE, FOR FOUR MASSACHUSETTS COMMUNITIES.

## Andover—1901.

MONTH	FREE AMMONIA	ALBUMINOID AMMONIA			CHLORINE	OXYGEN CONSUMED	BAC- TERIA
		Total	Soluble	Suspended			
January ...	.80	.43	.65	.17	.97	.51	1.21
February..	.75	.98	.26	1.84	1.11	.72	.87
March.....	1.02	1.10	1.18	1.00	.98	1.47	1.47
April.....	.73	.81	1.16	.39	1.02	.98	.87
May.....	.80	1.17	.99	1.38	.87	1.16	1.26
June.....	.90	.93	1.15	.68	1.20	.77	.66
July.....	1.00	1.57	.87	2.40	1.02	1.51	.25
August....	.70	.40	.52	.25	.70	.50	.02
September.	1.00	.50	.78	.17	.95	.53	.31
October...	1.36	1.21	1.39	1.00	1.07	1.25	1.52
November.	1.57	1.74	.83	1.63	1.20	1.63	2.89
December.	1.18	1.17	1.26	1.06	.90	1.01	.68

## Worcester—1899-1903.

January ...	1.01	1.06	1.18	1.00	.99	1.15	....
February..	1.05	1.06	1.27	.94	.92	1.14	....
March.....	.75	.84	.89	.82	.76	.86	....
April.....	.64	.66	.67	.65	.74	.82	....
May.....	.87	.81	.82	.84	.88	.85	....
June.....	.93	.91	.84	.91	.98	.88	....
July.....	.95	1.04	.95	1.10	1.17	.99	....
August....	.94	1.04	1.00	1.07	1.17	.99	....
September.	1.32	1.24	1.25	1.12	1.33	1.05	....
October...	1.14	1.19	1.05	1.28	1.14	1.14	....
November.	1.21	1.23	1.14	1.29	1.09	1.20	....
December.	.82	.94	.96	.93	.84	.91	....

TABLE 15—Continued.

Brockton—1900-1902.

MONTH	FREE AMMONIA	ALBUMINOID AMMONIA			CHLORINE	OXYGEN CONSUMED	BACTERIA
		Total	Soluble	Suspended			
January ...	1.60	.99	.95	1.02	.84	.83	....
February ..	.93	.89	1.03	.80	.78	.90	....
March .....	.81	.67	.94	.56	.67	.84	....
April .....	.89	.78	.84	.75	.77	.79	....
May .....	.85	.93	1.00	.90	.79	.87	....
June .....	.80	1.00	.93	1.05	1.06	1.99	....
July .....	.99	1.02	1.09	.91	1.47	1.16	....
August .....	.96	.98	.91	1.01	1.42	1.07	....
September.	1.30	1.38	1.18	1.53	1.43	1.27	....
October ...	1.21	1.33	1.11	1.48	1.08	1.25	....
November .	1.21	1.17	1.13	1.17	.89	1.13	....
December .	.94	.85	.92	.81	.86	.88	....

Lawrence—Sewage at Experiment Station—1901.

January ...	1.21	1.25	1.40	1.12	.79	1.20	.62
February ..	.99	1.06	1.12	1.02	.62	1.12	8.00
March .....	.80	1.12	1.10	1.14	.64	1.41	.52
April .....	1.02	.97	1.14	.80	.89	.95	.50
May .....	.81	.74	.73	.75	.97	.80	.30
June .....	1.03	.90	1.00	.80	1.28	.93	.48
July .....	.79	.76	.80	.73	1.36	.80	.23
August ....	1.14	.91	.87	.96	1.03	.83	.09
September.	1.06	.77	.66	.89	1.37	.80	.11
October ...	1.14	1.18	1.00	1.33	1.20	1.07	.14
November .	1.24	1.44	1.34	1.53	1.24	1.31	.17
December .	.75	.89	.91	.89	.62	.80	.14

## THE AVERAGE COMPOSITION OF BOSTON SEWAGE.

In estimating the strength of a sewage to be purified, both hourly and monthly fluctuations must be taken into account. The error due to seasonal variations is ordinarily eliminated by a series of analyses of day samples covering the entire year; and a correction for diurnal variation may be made with sufficient accuracy by applying a factor obtained from a series of hourly examinations. In the case of Boston sewage we have seen that analyses made between 10 A. M. and 1 P. M. bear to the average for the day the relation of 1.25 to 1.00. In the last line of Table 13 the corrected average of our daily analyses has been divided by the factor 1.25; and we believe that this fairly represents the general composition of the sewage passing the station at Albany

Street. In our daily analyses the months of January, February, and March are omitted and the results are so far imperfect; but since in the results for other cities, summarized in Table 15, these three months taken together do not depart far from the yearly average, we are inclined to believe this error not a very serious one.

To check this general representative analysis we have compared it in Table 16 with the straight average of the results of the six 24-hour examinations; and the agreement is seen to be close enough for any practical purposes. The representative analysis must of course be nearer the truth than the average of the daily samples of day flow or the average of six days, necessarily subject to individual variation.

TABLE 16.  
BOSTON SEWAGE—AVERAGE COMPOSITION OBTAINED BY VARIOUS METHODS.

	NITROGEN AS						OXY- GEN CON- SUMED
	Albuminoid NH <sub>3</sub>			Free NH <sub>3</sub>	Ni- trites	Ni- trates	
	Total	Diss.	Susp.				
A. Average of daily analyses.	6.2	3.1	3.1	19.9	.26	.15	45
B. Average of six 24-hour runs	4.8	2.6	2.2	12.5	.09	.68	42.9
Representative analysis cor- rected by factor for diurnal variation .....	5.0	2.5	2.5	15.9	...	...	36

Figures for nitrites and nitrates cannot, of course, be corrected by the factor 1.25, since their diurnal distribution is quite different. It will be noted that the nitrates were more than four times as high in the 24-hour runs as in the regular day analyses, while the nitrites were much lower. This is explained by the fact that nitrates are most abundant during the night hours, when the sewage contains free oxygen. During the day, when organic matter is present in large amount, the nitrates are reduced, appearing in part as nitrites.

For purposes of comparison we have prepared Tables 17-19, showing the composition of sewages in a number of English, American, and German cities. The analyses are of differing value, some representing only a few samples, some a long series, the



data upon which they are based being stated in footnotes. In the case of Lawrence, analyses are available for a considerable series of years, and in Table 20 we have cited the figures obtained at five-year intervals in order to illustrate the increase often noticeable in the strength of sewage from year to year.

TABLE 17.  
COMPOSITION OF SEWAGES—ENGLISH CITIES.  
Parts per Million.

CITY	SOLIDS		NITROGEN AS				OXYGEN CON- SUMED 4 HRS. AT 80° F.	CHLO- RINE
	Total	Soluble	Free NH <sub>3</sub>	Organic Nitrogen (Kjel- dahl)	Alb. Ammonia			
					Total	Soluble		
Huddersfield . . . . .	1061.	715.	10.7	—	6.0	—	99.0	123.
Leeds . . . . .	1680.	1334.	23.6	—	11.3	—	126.0	—
Leicester . . . . .	1680.	1045.	—	—	14.2	—	107.0	—
London (North) . . . . .	1353.	870.	39.0	—	4.3	—	80.0	132.
London (South) . . . . .	1501.	1093.	34.5	—	4.3	—	67.0	267.
Manchester . . . . .	1310.	940.	24.0	—	6.0	—	118.0	170.
Belfast . . . . .	1848.	143.	21.3	15.3	8.9	—	90.1	679.
Sutton . . . . .	1628.	804.	114.3	—	8.85	—	57.1	114.
Aylesbury . . . . .	1191.	824.	68.4	—	7.8	—	57.4	123.
Blackburn . . . . .	1076.	602.	28.2	—	3.4	—	35.8	81.
Exeter . . . . .	544.	299.	37.7	—	2.1	—	20.2	50.
Saltley . . . . .	2190.	1455.	41.6	39.1	15.6	—	134.3	125.
Rea . . . . .	1886.	1340.	35.0	48.3	15.2	—	173.8	245.
Hockley . . . . .	2155.	1451.	33.7	59.7	16.7	—	140.4	216.
Aston . . . . .	1832.	948.	51.7	92.8	19.5	—	133.3	203.
Dublin . . . . .	1523.	1190.	32.4	—	40.1	—	87.8	201.

Huddersfield.—CAMPBELL, K. F., 1900, 1902. Average of five 24-hour tests with mean analysis of sewage from Aug. 1898 to Feb. 1900. Sewers on the combined plan.

Leeds.—HARDING, 1902. Mean analyses from Nov. 1898 to June 1900. Sewers on combined plan.

Leicester.—MAWBEY, E. G., 1903. Average of analyses made from Sept. 1898 to Sept. 1899. Combined plan.

London—Northern Outfall.—DIBDIN, 1884. Average analyses for 1893. CRIMP, S., 1893. Average analyses for 1893. DIBDIN, 1893. Average analyses for 1894. CLOWES, 1902. Sept. 1898, April 1899, Nov. 1900 to Aug. 1901.

London—Southern Outfall.—DIBDIN, 1893. Average for years 1893 to 1894. CLOWES, 1902. Nov. 1899 to July 1900.

Manchester—MANCHESTER, CITY OF, 1901. Period from May 16, 1900, to Jan. 24, 1901. Four daily samples per week, made up of hourly samples, which were mixed in equal portions.

Belfast.—LETTA, 1903. Twelve analyses, Jan. to June 1902.

Sutton.—DIBDIN, 1902. Twenty-seven analyses, Feb. 1897 to Aug. 1898,

Aylesbury.—DIBDIN, 1902.—Fifteen analyses.

Blackburn.—DIBDIN, 1902. Seventeen analyses.

Exeter.—DIBDIN, 1903. Twelve analyses.

Saltley.—REA, HOCKLEY AND ASHTON, WATSON, 1903. One analysis each.

Dublin.—CAMERON, 1903.

TABLE 18.  
COMPOSITION OF SEWAGE—CONTINENTAL CITIES.  
Parts per Million.

CITY	SOLIDS		NITROGEN AS						OXY.CONSUMED		CHLO- RINE
	Total	Soluble	Free NH <sub>3</sub>	Organic N		Alb. NH <sub>3</sub>		Total	Soluble		
				Total	Soluble	Total	Soluble				
(a) Halle. .	3388.	2794.	89.	97.	59.					715.	
Breslau . .	1178.	773.	73.							182.	
(b) Halle. .	2458.	1633.		55.	21.					209.	
(a) Berlin.		695.	60.						294.	164.	
Hamburg	1133.	922.	35.			5.06		54.	290.	312.	
Cristiania	1097.	639.	19.	31.				62.		167.	
Paris. . . . .	630.		16.							50.	
Zürich . . .	607.	480.	8.	33.	18.					22.	
(b) Berlin.	2172.	1088.	*	*						264.	
Frankfurt	2062.	898.	63.	54.	11.			126.	—	30.	

\* Nitrogen as Free NH<sub>3</sub>+Soluble Organic N (by Kjeldahl) = 108.8.

Halle (a).—3 analyses. KÖNIG, 1887. With water closets.

Halle (b).—5 analyses. KÖNIG, 1887. No water closets.

Breslau.—Average 72 analyses. KÖNIG, 1887.

Berlin (a).—Average 14 analyses. BRUCH, 1899.

(b).—30 analyses. KÖNIG, 1898.

Hamburg.—Average of analyses from Sept. 1898 to March 1899. DUNBAR, 1899. Oxygen consumed by Kubel method.

Cristiania, Norway, 1902. Average 41 analyses during 1900-1901.

Paris.—Single analysis, 1900. STEURNAGLE, 1900; HOLST, 1902.

Zürich.—4 analyses. KÖNIG, 1887. No water closets.

Frankfort, 1891. Eight analyses. LEPSIUS, 1891.

TABLE 19.  
COMPOSITION OF SEWAGES—AMERICAN CITIES.  
Parts per Million.

CITY	SOLIDS		NITROGEN AS			TOTAL OXYGEN CONSUMED	CHLO- RINE
	Total	Soluble	Free Am- monia	Alb. Ammonia			
				Total	Soluble		
Brockton, Mass.....	482.	381.	2.9	5.36	3.43	80.7	83.0
Framingham, Mass.....	1031.	364.	21.9	8.46	3.17	97.9	64.2
Gardner, Mass.....	508.	240.	24.7	8.12	3.18	57.3	42.0
Leicester, Mass.....	747.	602.	29.3	6.86	3.73	21.6	56.2
Marlborough, Mass.....	520.	341.	33.3	6.68	3.19	58.8	60.7
Medfield, Mass.....	825.	696.	9.6	7.40	5.93	67.3	87.8
Natick, Mass.....	349.	297.	12.5	2.58	1.59	29.3	57.0
Spencer, Mass.....	293.	168.	13.4	3.91	1.61	32.9	30.8
Newport, R. I.....	—	—	17.2	8.14	—	59.9	164.7
Plainfield, N. J.....	530.	374.	31.7	12.8	—	58.25	49.6
Worcester, Mass.....	870.	553.	15.7	5.97	2.23	93.0	80.9
Berlin, Ont.....	—	—	20.9	16.74	—	499.5	61.0

## DATA FOR TABLE 19.

Brockton, Framingham, Gardner, Leicester, Marlborough, Medfield, Natick, Spencer.—

Average of 12 monthly analyses during 1899. MASS. STATE BOARD OF HEALTH, 1900.

Newport, R. I.—Average of daily analyses from June 2 to Oct. 13, 1894.

Plainfield, N. J.—By ROLPH.

Worcester, Mass.—Average of 48 monthly averages of weekly analyses of sterilized daily samples, during 1900-1903 inclusive. The total solids are for the year 1901 only.

EDDY, 1900-1903.

Berlin, Ont.—Average of daily analyses from June 26 to Sept. 4, 1902. AMYOT, 1902.

TABLE 20.

YEARLY AVERAGE ANALYSES OF SEWAGE AT LAWRENCE, MASS.

Parts per Million.

YEAR	FREE AMMONIA	ALBUMINOID AMMONIA		OXYGEN CONSUMED (Kubel, 2 Min.)	BACTERIA PER 1 C.C.
		Total	Soluble		
1888	15.5	6.8	1.6	—	1,000,000
1893	26.8	6.3	3.1	34.5	923,000
1898	29.9	5.8	2.9	32.2	1,862,000
1902	44.9	7.2	3.1	40.6	3,377,400

Compiled from the Annual Reports of the Massachusetts State Board of Health.

Table 19 shows that in American sewages the free ammonia ranges from 3 to 30 parts and averages somewhere near 15 parts; figures for albuminoid ammonia vary from 2 to 15 parts and average near 7; oxygen consumed analyses lie between 20 and 100 parts and average about 60. Judged by these standards, Boston sewage is seen to be relatively strong in free ammonia and weak in albuminoid ammonia and oxygen consumed. The European sewages appear to be several times as strong as the American sewages in all constituents.

In Table 21 we have calculated the total amount of the various constituents in Boston sewage, multiplying the average daily analyses in Table 13 by the flow obtained from the pump records at Dorchester. We have assumed that the flow at Albany Street is 55 per cent of the total pumpage at Dorchester for the reasons previously cited, and the first column of Table 21 thus represents the estimated flow past the experiment station. The other figures are obtained by multiplying the analytical data in Table 13 by the estimated flow. The totals for the nine months are corrected by dividing by the factor 1.25; and finally the results are expressed in grams per capita per day.

TABLE 21.

## BOSTON SEWAGE.

Total Amount of Various Constituents.

DATE	FLOW BILLIONS OF LITERS	THOUSANDS OF KILOGRAMS				
		Nitrogen as				Oxygen Consumed Total
		Albuminoid Ammonia			Free NH <sub>3</sub>	
		Total	Soluble	Suspended		
April, 1904.....	8.3	43	23	20	130	340
May, 1904.....	8.8	50	22	28	130	270
June, 1904.....	7.9	55	24	31	160	340
July, 1903.....	7.1	44	23	21	130	330
August, 1903.....	6.9	49	26	23	150	310
September, 1903..	7.1	44	21	23	150	320
October, 1903....	7.9	50	26	24	160	400
November, 1903...	7.9	48	23	25	180	420
December, 1903...	8.8	58	32	26	210	440
Total, Nine Months .....	70.7	441	220	221	1,400	3,170
Total corrected for Diurnal Variation ....		352	176	176	1,120	2,536
Grams per Capita Daily .....		3.7	1.8	1.8	12.	27.

The values obtained are higher than those given for any other American sewage with which we are acquainted. Fuller (1903) offers as approximate average amounts of the various constituents in grams per capita daily, 15 for oxygen consumed, 7 for free ammonia, and 2.5 for albuminoid ammonia. Our figures are respectively 27, 12, and 3.7, from 55 per cent to 70 per cent higher. The analysis of Boston sewage as we have seen corresponds well with that of other American cities; its higher per capita content is due to the fact that its per capita flow is higher. The total amount of sewage pumped at Dorchester from July, 1903, to June, 1904, inclusive, was 46,000,000,000 gallons, which, with a contributing population of 477,000, makes a daily per capita flow of 264 gallons. Even using the total population of the South Metropolitan District, 669,000, we get a daily per capita flow of 188. Fuller's figures were all much lower than this,



the per capita flow at Framingham being 73, at Gardner 100, at Marlborough 113, and Worcester 115. The per capita flow appears to increase with the size of the community, as is the case with the per capita consumption of water, and it is natural that a city like Boston, with a considerable transient population, should exhibit this phenomenon to a marked degree.

It is at any rate evident that in dealing with cities of the first rank our estimates of per capita yield of polluting material must be materially modified; and we gain a new idea of the vastness of a problem which involves the treatment of 1,400,000 kilograms of nitrogen in the form of free ammonia in one only of the principal sewers of a city in a single year.

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THE NUMBER OF BACTERIA IN SEWAGE AND SEW-  
AGE EFFLUENTS DETERMINED BY PLATING  
UPON DIFFERENT MEDIA AND BY A  
NEW METHOD OF DIRECT  
MICROSCOPIC ENU-  
MERATION.

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Massachusetts Institute of Technology.)*

INTRODUCTION.

THE main object in sewage purification is the oxidation or removal of its organic constituents to such an extent that the effluent shall not be subject to putrefactive decomposition. The aim of the process is therefore chemical and its success is gauged by chemical methods. The bacteriology of sewage and sewage effluents is, however, of importance since a knowledge of the bacteria which are the chief agents in the destruction of organic matter must make it easier so to adjust conditions as to obtain the maximum possible results. The study of the nitrifying bacteria, for example, has already yielded valuable data as to the effect of various chemical and physical agents upon these organisms. The admirable papers by Boullanger and Massol (1903), and by Schultz-Schultzenstein (1903), the latter translated into English by Kimberly (1904), are models of thorough and accurate investigation. The third paper of recent date on the same subject, by Fremlin (1903), fails to carry the conviction that errors of manipulation have been successfully avoided.

The bacteria active in the newer processes of sewage purification, in the septic tank and in the contact and trickling filters, have as yet received almost no attention; and it is hoped that the work planned at the Sanitary Research Laboratory of the Massachusetts Institute of Technology may to some extent supply this deficiency during the next few years. Investigations should be

made which are at the same time quantitative and qualitative, and they ought ultimately to include the detailed study of all the forms isolated from a series of plate cultures. I have thought that the ground might be cleared to some extent by quantitative analyses alone, so carried out under different conditions as to measure roughly certain principal groups of bacteria. With this end in view the following preliminary studies were undertaken.

#### PREVIOUS QUANTITATIVE WORK ON SEWAGE BACTERIA.

Determinations of the total number of bacteria present in sewage were made nearly 20 years ago by Wahl (1886), who found from 1,686,000 to 5,248,000 bacteria per c.c. in the sewage of Essen. Miquel (1891), records 13,800,000 per c.c. in the sewage at Gennevilliers with an average of 7,475 in the effluent from the broad irrigation fields. E. Ray Lankester (1892), at Oxford, England, found an average of 3,170,000 bacteria in sewage and 40,000 per c.c. in the effluent from a sewage farm. The Franklands (1894), note the presence of 26,000,000 in the sewage outlet at Ballater in Scotland. Laws and Andrewes (1894), record from two to eleven million bacteria in various London sewages.

It is probable that certain of these early experimenters did not appreciate the necessity for planting samples promptly to forestall the enormous multiplication which takes place soon after collection. Fuller (1895), and Winslow and Belcher (1904), have shown that a tenfold increase may take place under such conditions in 24 hours. In recent years English investigators, as shown in Tables 3 and 4 have found from two to five million bacteria per c.c. In Germany, according to Bruch (1899), the sewage of Berlin showed, in one series of analyses, 1,600,000 bacteria per c.c. and in another from 600,000 to 3,800,000. Examinations at Charlottenburg quoted by Grünbaum (1900), showed 2,700,000 bacteria in crude sewage and 225,000 in contact effluents.

In America the reports of the Massachusetts State Board of Health show that the sewage of Lawrence taken from the Lawrence Street sewer contained on the average from 1894 to 1901, 2,800,000 bacteria per c.c. Each year the Lawrence Experiment Station furnishes an immense accumulation of valuable data published in less detail in the last two reports than formerly. The monthly analyses of Lawrence and Andover sewage are extracted from the Report for 1900 to form the first two columns of Table 1; and Table 2 is compiled from the same volume (Clark, 1901), to show the total number of bacteria present in effluents of various types.

The third column of Table 1 is from a report on the Worcester purification plant by Eddy (1902); the yearly averages reported by the same author are as follows: Sewage, 3,712,000; septic tank effluent, 2,539,000; sand filter effluent, 41,900.

Analyses of American sewage outside the Massachusetts Reports are, unfortunately, rare. At Plainfield, N. J., Le Clear (1902) records the following average numbers of bacteria per c.c.: Crude sewage, 1,321,000; septic tank effluent, 556,800; contact filter effluent, 171,500. At Ames, Iowa, Walker (1901) and Pammel (1902) have carried out somewhat exhaustive studies.



# NEW METHOD OF ENUMERATING SEWAGE BACTERIA 211

Their monthly results form the fourth column of Table 1; the general averages for the year 1901 are as follows: Sewage, 1,248,256; septic tank effluent, 991,298; sand filter effluent, 14,750.

TABLE 1.  
BACTERIA IN SEWAGE. MONTHLY AVERAGES. BACTERIA PER C.C.  
(Clark, Eddy, Walker.)

Month	Lawrence 1900	Andover 1900	Worcester 1901	Ames 1901
January.....	2,860,000	3,494,000	5,237,000	550,879
February.....	1,520,000	4,475,000	7,667,000	1,993,766
March.....	1,814,000	2,260,000	5,559,000	469,600
April.....	2,320,000	9,963,000	6,400,000	775,090
May.....	2,334,000	5,150,000	2,457,000	652,150
June.....	2,530,000	4,890,000	3,614,000	825,333
July.....	3,150,000	4,235,000	3,987,000	826,000
August.....	2,645,000	3,153,000	2,531,000	1,194,000
September.....	6,485,000	1,100,000	3,390,000	940,000
October.....	3,178,000	4,253,000	1,968,000	4,230,000
November.....	1,710,000	3,850,000	2,937,000	1,547,000
December.....	4,060,000	4,277,000		3,825,000

TABLE 2.  
AVERAGE BACTERIAL ANALYSES OF EFFLUENTS FROM VARIOUS TYPES OF  
FILTERS AND SEPTIC TANKS DURING 1900, AT ANDOVER  
AND LAWRENCE, MASS. BACTERIA PER C.C.

Effluent from Septic Tanks	Effluent from Contact Filters	Effluent from Trickling Filters	Effluent from Sand Filters
1,209,500	94,500	74,200	73,900
1,929,000	486,200	69,700	1,175
	552,100		10,072
	386,400		1,243
	291,800		152
	543,900		10,300
	630,000		6,500
			16,300
			25,600
			1,485
			24,200
			252
			151
			23,600
			16,700
			4,700

So far only the total number of bacteria present has been considered; but in the various reports published by the Royal Sewage Commission of Great Britain and by the London County Council analyses are presented which go into greater detail. Woodhead (Rideal, 1899) found in Exeter

sewage, from 3,000,000 to 5,000,000 non-liquefying and 500,000 liquefying bacteria on aerobic plates and in anaerobic cultures, 300,000 liquefiers and 700,000 non-liquefiers. Klein and Houston (1899) report an average of 3,600,000 bacteria in sewage, of which 460,000 belonged to the *B. coli* group, with 1,300 anaerobic spore formers. Clowes (1898) gives the following figures for London sewage (Table 3).

TABLE 3.  
BACTERIA IN LONDON SEWAGE. NO. PER C.C.  
(Clowes.)

Source of Sample	Date	Total No. of Bacteria (Gelatin 20°)	B. Coli and Closely Allied Forms	No. of Spores	Liquefiers	Spores <i>B. subtilis</i> <i>B. mesentericus</i> <i>B. mycoides</i> <i>B. megatherium</i>	<i>B. fluorescens</i> Liquefactans
Barking	Feb. 23 to May 4, '98	4,399,047	70,000	690	357,500	20	25,000
Crossness	Feb. 23 to May 2, '98	3,526,669	112,500	852	404,000	20	10,000

Rideal (1901) quotes figures for several other cities. At Exeter the crude sewage contained three to five million bacteria with 150,000 to 200,000 liquefiers, the tank effluent, one million with 300,000 to 400,000 liquefiers, and the final filter effluent, 900,000 bacteria with 100,000 liquefiers. At Chorley the crude sewage contained four million bacteria, the tank effluent 400,000, and the filter effluent, 46,000. At Leeds the crude sewage contained 2,500,000 to 3,000,000 bacteria per c.c. More exhaustive studies on London sewage and contact filter effluents were published by Clowes and Houston (1899 and 1903) in the second and third reports to the London County Council; these are summarized in Table 4.

TABLE 4.  
BACTERIA IN LONDON SEWAGES AND EFFLUENTS. NO. PER C.C.  
(Clowes and Houston.)

SEWAGE.							
Source of Sample	Date	Total No. of Bacteria (Gelatin 20°)	B. Coli and Closely Allied Forms	Anaerobic Spores (Agar 37°)	Aerobic Spores	Liquefiers	Total No. of Bacteria (Agar 37°)
Barking .....	Oct. 16, '99, to Jan. 17, '00.	7,096,666	770,000				
" .....	Mar. 16, '99.	10,000,400	600,000				
Crossness .....	May 11, '98, to Dec. 21, '98.	7,357,692	600,000				
" .....	Jan. 11 to Feb. 22, '99.			554	340	1,076,923	
" .....	Mar. 22 to Oct. 4, '99.	5,711,000	655,555				
" .....	Aug. 2 to Oct. 4, '99.	5,558,571					2,802,857

TABLE 4—Continued.

## CONTACT BEDS.

Source of Sample	Date	Total No. Bacteria (Gelatin 20°)	B. Coli and Closely Allied Forms	Anaerobic Spores (Agar 37°)	Aerobic Spores	Liquefiers	Total No. of Bacteria (Agar 37°)
Barking .....	Oct. 16, '99, to Jan. 17, '00.						
Bed A							
Primary ..		2,180,000	500,000				
Bed B							
Primary ..		2,700,000	200,000				
Bed A							
Secondary		1,918,571	200,000				
Bed B							
Secondary		1,444,285	233,500				
Barking.....	Mar. 16, '99.						
Fine rag stone bed.....		4,000,000	500,000				
Fine coke bed.....		1,800,000	300,000				
Crossness .....	May 11, '99, to Dec. 20, '00.						
4 ft. coke bed.....		4,966,666	400,000		252	806,666	
6 ft. primary coke bed.....		6,787,500	600,000		256	837,500	
6 ft. secondary coke bed.....		4,300,000	100,000				
4 ft. coke bed.....	Jan. 11, '99, to Feb. 23, '99.				320	833,333	
6 ft. primary coke bed.....				342			
6 ft. secondary coke bed.....				354			
13 ft. coke bed.....				207			
13 ft. coke bed.....	Mar. 22 to Oct. 4, '99.	5,364,000	411,111				
	Aug. 2 to Oct. 4, '99.	4,662,857					2,802,857

The sewage at West Derby and Walton, examined by Boyce (1900), showed the following results: Walton sewage, 13,400,000 bacteria per c.c.; West Derby sewage, 10,380,000; West Derby contact filter effluent, 614,000 (of which 10,000 were of the *B. coli* group and 100 anaerobic spore formers); West Derby sand filter effluent, 17,900 (of which 50 belonged to the *B. coli* group). Boyce, MacConkey, Grünbaum and Hill (1902) report the following results from the same locality (Table 5).

 TABLE 5.  
 BACTERIA IN EFFLUENTS AT WEST DERBY. NO. PER C.C.  
 (Boyce, MacConkey, Grünbaum, and Hill.)

Source of Sample	Total Bacteria	B. Coli Group	Anaerobic Spores
Contact Filter.....	838,000	9,800	10-100
Sand Filter 1.....	79,800	125	Less than 1
2.....	50,700	590	Less than 1
3.....	31,700	49	Less than 1
4.....	113,800	432	Less than 1

Lorrain Smith (1903) presented to the Royal Commission on Sewage Disposal analyses of sewage and effluents at Belfast which indicated considerably higher numbers than those elsewhere recorded. His chief results are averaged and brought together in Table 6.

TABLE 6.  
BACTERIA IN BELFAST SEWAGE. NO. PER C.C.  
(Smith.)

Source of Sample	Total No. Bacteria (Gelatin 20°)	Liquefiers	Anaërobes	Aërobic Spores	B. Coli
Crude sewage.....	15,300,000	1,510,000	4,000,000	86	300,000
Screened and sedimented sewage.....	47,280,000	2,860,000	19,000,000	143	400,000
Effluents from primary contact beds.....	35,660,000	1,700,000	12,700,000	76	400,000
Effluents from secondary contact beds.....	21,850,000*	1,120,000	9,680,000	62	200,000

With regard to certain groups, of alleged significance in sanitary water analysis, further data are available. Klein and Houston (1898, 1899) report in crude sewage from 30 to 5,000 spore-bearing anaërobes per c.c., and 90,000 to 2,000,000 organisms of the B. coli group. According to Houston (1899, 1902) crude sewage contains over 10 million bacteria per c.c., as determined by plating on gelatin, between one and ten million on agar at 37°, 100,000 organisms of the B. coli group, at least 1,000 sewage streptococci, and 1,000 to 10,000 anaërobic spore-formers. A recent investigation by Belcher and the writer (Winslow and Belcher, 1904), indicated somewhat smaller numbers of all these groups in American sewage. Samples were taken from a small lateral of the Boston system receiving very fresh domestic sewage, and the results might thus be expected to differ from those obtained in the London experiments. Anaërobic spores were found present in numbers less than 1,000 per c.c.; the B. coli group amounted to 28,000 per c.c. In these experiments all the colonies found upon dilute plates were fished and worked out in sufficient detail to place them in certain general groups whose characteristics are given in the original paper. The distribution in fresh sewage is shown in Table 7; the authors found in sewage stored in a glass-stoppered bottle that the bacteria of all groups multiplied tenfold within 24 hours, and then began to decrease.\*

TABLE 7.  
BACTERIA IN FRESH BOSTON SEWAGE.  
(Winslow and Belcher.)

BACTERIA PER C.C.										
On Gelatin at 20°			Lactose Agar at 37°				Anaërobic Agar			
1,240,000			151,000				140,000			
Group.....	II	IV	Va	VIII	X	XI	XII	XIII	XIV	XV
Type.....	Cocci	Chromogenes	B. subtilis	B. liquidus	B. coli	B. typhi	B. candidans	B. aërogenes	B. ubiquitus	B. rhinocleromatis
No. per c.c.	372,000	128,000	74,500	30,000	28,000	60,000	44,000	30,000	162,000	154,000

\*For assistance in the collection of the foregoing references the author is indebted to Mr. G. C. Bunker.



## SOURCE OF SAMPLES EXAMINED.

The Sanitary Research Laboratory of the Massachusetts Institute of Technology is situated near the junction of Albany street and Massachusetts avenue on the south side of Boston and on the line of the nine-foot main trunk sewer of the Boston Main Drainage Works. This is the principal vein of the South Metropolitan system receiving the sewage of Boston proper, Roxbury, Brighton, Allston, Newton, Brookline, Watertown, and Waltham, and its contributing population is over 300,000. At the station some 10,000 gallons a day are pumped from this sewer into three supply tanks  $6 \times 4 \times 3$  ft. deep, from which it flows by gravity to the various experimental tanks and filters. As a rule, samples of the sewage were taken as it flowed from these supply tanks. Further statistics, with detailed monthly and hourly analyses of the station sewage will be found in another communication (Winslow and Phelps, 1905).

The septic tanks used in these experiments were cypress tanks,  $6 \times 4 \times 4$  ft. deep. Four of them (5, 6, 8, 10) are closed tanks, and were first put in operation in June, 1903. Tank 6 is filled with  $1\frac{1}{2}$ -inch broken stone. Tanks 7 and 9 are open tanks started in February, 1904. The contents of Tanks 5, 6, and 7 are changed once in 12 hours, of Tanks 9 and 10 once in 24 hours, and of Tank 8 once in 48 hours.

The contact filters studied are tanks  $4 \times 4 \times 6$  ft. deep, or 4 ft. deep in the case of Nos. 17 to 20. No. 11 is filled with 2-3 in. coke; No. 12 with  $1\frac{1}{2}$ -in. crushed stone; Nos. 13 and 16 with  $\frac{1}{2}$ -in. crushed stone; No. 14 at first with 1-in. stone, later with tile-bricks arranged in regular open tiers. All these are single-contact filters receiving crude sewage in doses ranging from one to two million gallons per acre per day. Tanks 19 and 20 are primary contact filters of  $1\frac{1}{2}$ -in. stone, and 17 and 18 are secondary beds of  $\frac{1}{2}$ -in. stone. Seventeen and 19 receive septic sewage, 16 and 18 raw sewage at rates of one to two million gallons per acre per day for each bed. All were put in operation in June, 1903.

The trickling filters of the station are tanks  $4 \times 4 \times 6$  ft. deep filled with  $1\frac{1}{2}$ -in. crushed stone, and dosed by tipping buckets at a rate of 1,500,000 to 3,000,000 gallons per acre per day. Tank 15 takes sewage which has been septicized for 12 hours; Tank 23, septicized for 48 hours; and Tank 22, raw sewage. No. 15 was put in operation in July, 1903, and Nos. 22 and 23 in February, 1904.

The sand filters used are tanks  $6 \times 4 \times 3$  ft. deep filled with 2 ins. of sand of effective size, .17 mm., resting on 6 ins. of coarser material. Tank 1 received 100,000 gallons of raw sewage per acre per day from June 30 to December, 1903, 200,000 gallons per acre per day for the first six months of 1904, and 400,000 gallons since June 24, 1904. Tanks 24 and 25 have received 400,000 gallons of septic sewage per acre per day since February, 1904. The sewage applied to No. 24 has been septicized for 24, that applied to 25, for 48 hours.

The bacteriological analyses were carried out in two series, one extending from July to December, 1903, and including examinations made twice a month, and the second comprising weekly analyses made in July and August,

1904. These are designated in the appended table as Series A and Series B respectively. In the analyses of Series A, I was assisted by Professor S. C. Prescott and Mr. E. B. Phelps, to whom I desire to express my thanks.

#### THE DIRECT MICROSCOPIC ENUMERATION OF BACTERIA.

Realizing that the ordinary culture methods reveal only a fraction of the bacteria present, I attempted to control them by a direct examination of sewage and effluents under the microscope, drying a measured volume upon a cover-slip of known area, and counting representative fields with a Sedgwick-Rafter micrometer such as is used for enumerating the larger micro-organisms in the examination of drinking water. In my first experiments I attempted to stain the bacteria in the liquid in which they were suspended by adding a few drops of methylene blue or gentian-violet to a one-ounce sample bottle of sewage. Methylene blue was soon discarded, because bacteria grew in the stain itself, and later carbol-fuchsin was substituted for gentian-violet because it was found to give larger counts. Finally the process of staining in the bottle was entirely abandoned. Five hundredths c.c. of the fresh sample was placed on the cover-slip, dried in the air, fixed in the flame, and stained with carbol-fuchsin by heating till steam appeared. The latter process stained the cells much more definitely and sharply, and gave higher and more constant results than those obtained by staining in the bottle. It is of interest to note that on many of the slides prepared by the earlier method some of the bacteria showed faint but unmistakable flagella, stained by the carbol-fuchsin without any mordant; and it is possible that some process by which staining reagents are added directly to liquid cultures in which bacteria are present in their most active state might give better results than the somewhat severe preliminary drying and fixing treatment to which they are usually subjected before staining.

The method of direct enumeration as finally developed offered no serious technical difficulties, and furnished constant and comparable results. Its accuracy and the significance of its results have been investigated more fully by Willcomb and myself in another communication (Winslow and Willcomb, 1905). It will there be shown that there are three main factors which might tend to make the microscopic count larger than the plate count; the inclusion of several bacteria in a single colony, the presence in the sample of dead bacteria in a stainable condition, and the presence of organisms which do not grow on our nutrient media. We shall show that with pure cultures of ordinary metatrophic bacteria the plate counts and microscopic counts closely correspond even when the number of bacteria present are rapidly decreasing. One hundred million cells per c.c. have disappeared in four hours without leaving any trace of stainable bacteria. It

appears, therefore, that the presence of dead cells introduces no serious error in the microscopic count—that its excess is due mainly to the presence of organisms which fail to appear upon our plates—and that it, therefore, furnishes a more accurate measure of the total number of bacteria present than do our ordinary methods.

If these conclusions are justified the results shown in Tables 8 to 12 represent the first attempt to determine the actual extent of the bacterial flora of sewage. The crude sewage itself contained on an average 29 million bacteria per c.c.; the septic tank effluents, 30 million; the contact filter effluents, 24 million; the trickling filter effluents, 17 million; and the sand effluents, 650,000. The ratio of the total number as determined by direct microscopic enumeration to the count upon gelatin plates was nearly 20 in the case of sewage, about 40 for the septic tanks and contact and trickling filters, and 70 for the sand filter effluents. This result is suggestive in view of the important rôle played in purification processes by the nitrifying organisms which are known not to develop upon our gelatin plates.

#### THE BACTERIA IN RAW SEWAGE.

The media used for these analyses included lactose gelatin, lactose agar and Nährstoff agar. The first two were made up according to the standard methods of the American Public Health Association, two per cent of lactose being added before the final filtration and the reaction adjusted to  $-0.5$  on Fuller's scale. The Nährstoff agar contained one per cent of agar and one per cent of Heyden's Nährstoff dissolved in water and filtered through cotton.

Anaërobic cultures were made first by the Wright method (Wright, 1901), later according to the admirable modification of Rickards (1904), by inverting a tube or Erlenmeyer flask containing the inoculated and solidified medium in a tumbler of pyrogalic acid and caustic solution. Cultures incubated at  $37^{\circ}$  were counted after 24 hours; gelatin plates and anaërobic cultures at  $20^{\circ}$  after 48 hours, and Nährstoff plates after seven days. Acid production was observed on plates to which litmus had been added in the usual manner, both agar and gelatin plates in Series A and gelatin plates in Series B.

The average results of the analyses of sewage are presented in Table 8 with the ratio which the count on each medium bears to the count on lactose gelatin at 20°.

TABLE 8.  
BACTERIA IN BOSTON SEWAGE.  
SERIES A.

NO. OF SAMPLES	BACTERIA PER C.C.					
	On Lactose Gelatin at 20°			On Lactose Agar at 37°		On Anaërobic Agar
	Liquefiers	Acid Formers	Total	Acid Formers	Total	
56	365,000	1,670,000	5,430,000	1,670,000	3,760,000	2,440,000
Ratio to Gelatin Count.....	7	31	100	31	69	45

SERIES B.

NO. OF SAMPLES	BACTERIA PER C.C.						
	Microscopic Count	Lactose Gelatin at 20°			Lactose Agar at 37°	Nährstoff	Anaërobic Gelatin
		Liquefiers	Acid Formers	Total			
25	29,000,000	149,000	429,000	1,690,000	1,400,000	2,930,000	850,000
Ratio to Gelatin Count..	1700	9	25	100	83	170	50

The total number of bacteria on lactose gelatin was three times as great in Series A as in Series B; this is due to the fact that the first series included the autumn months in which the number of bacteria in sewage reaches a maximum. In Table 9, the monthly values obtained in the first set of experiments show clearly this autumnal maximum which is also manifest in the Lawrence and Ames results of Table 1.

The average number of bacteria present in Boston sewage appears then to vary between 500,000 and 10,000,000 according to the season. These figures are, however, too high since they were obtained from samples collected during the daytime when the numbers are of course much higher than at night. Phelps and the writer have elsewhere (Winslow and Phelps, 1905), published



a series of hourly analyses which show the diurnal variation in the number of bacteria present to be very great. The numbers in Table 8 may, however, fairly be compared with those from other places which have generally been obtained in the same way by examination of day samples. They show that Boston sewage has the bacteriological composition which appears to be common to most of the European and American cities which have been examined. There is much less variation indeed than might have been expected. Of the localities for which bacteriological sewage

TABLE 9.  
BACTERIA IN BOSTON SEWAGE. BY MONTHS. NO. PER C.C.

MONTH (1903)	ON LACTOSE GELATIN AT 20			ON LACTOSE AGAR AT 37		ON ANAEROBIC LACTOSE AGAR AT 20°
	Acid Formers	Liquefiers	Total	Acid Formers	Total	
July .....	445,900	314,300	2,995,000	420,000	1,864,300	3,480,000
August .....	318,750	150,000	4,263,600	1,133,000	2,688,900	1,461,500
September....	4,021,900	850,000	11,487,500	3,268,750	8,504,400	4,557,500
October .....	635,000	95,700	3,693,000	1,298,300	1,407,000	639,300
November ....	530,400	15,000	587,100	530,600	551,300	605,100
December ....	655,000	8,700	712,000	762,000	814,000	696,000

analyses are quoted above, Essen, Berlin, Charlottenburg, Leeds, Exeter, Chorley, Oxford, Lawrence, Andover, Ames, Plainfield, Worcester, and Boston show results generally lying between 1 and 5 millions, London, Walton and W. Derby, figures varying from 2 to 10 millions, and Paris, Ballater and Belfast, over 10 millions. The accuracy of the latter results may be questioned since multiplication in storage so easily occurs.

In both series of analyses of Table 8 the ratios of the liquefiers and acid formers were remarkably constant. The liquefiers amounted to seven and nine per cent respectively of the total number of colonies on gelatin, a slightly lower figure than that obtained by English observers. Clowes (1898), found about 10 per cent of liquefiers; Woodhead at Exeter, about 10 per cent; Clowes and Houston (1903), 10 to 20 per cent; and Smith (1903), 1 to 15 per cent. The ratio of acid formers in Series A was 31 per cent both on gelatin at 20° and agar at 37°, and in Series B 25 per cent. It is significant to note the coincidence of these

figures which show that the acid forming organisms are in general so adapted to the body temperature that their counts at 37° are as large as at 20°, while the total number of bacteria on agar at 37° varies from 70 to 80 per cent of the total number on gelatin at 20°. Since in potable water the ratio of the 37 per cent count to the 20° count is generally under 10 per cent with acid formers absent, the application of the lactose agar plate to sanitary water analysis is apparent.

The anaërobic counts in Series A and B differ but little although made in one case on gelatin and in the other on agar. In each the anaërobic colonies were about half as numerous as the aërobic colonies on gelatin. In a few experiments with aërobic and anaërobic plates of Nährstoff the same general relation was found to hold.

Nährstoff agar incubated at 20° for seven days showed not quite twice as many bacteria as appeared on the gelatin plate in two days. This result is somewhat lower than that obtained by Gage and Phelps (1902), who report that with the count on Nährstoff agar as 100, that on gelatin was 34 after two days and 44 after three days when sewage was examined. With a sand effluent the gelatin count was less than 20 per cent and with river water, less than 10 per cent of the Nährstoff count.

#### BACTERIA IN SEPTIC TANK EFFLUENTS.

The analyses of the septic tanks studied, four in number in Series A, six in Series B, are summarized in Table 10; and for comparison the total numbers of bacteria on gelatin and the ratio to that number of the counts made in other ways are shown in Table 14 for all the various types of effluents examined.

The total number of bacteria shows a marked decrease after passing through the septic tanks, amounting to over 60 per cent in Series A and over 50 per cent in Series B. The Lawrence figures in Table 2 show a similar decrease compared with the sewage in Table 1; and at Worcester, Plainfield, and Ames the same phenomenon appears, the diminution amounting to about 50 per cent. At Exeter, Woodhead reported no such decrease. The ratios of the different groups to the total are almost identical with those obtained with crude sewage. Comparing the septic

TABLE 10.  
BACTERIA IN SEPTIC TANK EFFLUENTS.

SERIES A.

	No. OF SAMPLES	BACTERIA PER C.C.					
		On Lactose Gelatin at 20°			On Lactose Agar at 37°		On Anaërobic Agar
		Lique-fiers	Acid Formers	Total	Acid Formers	Total	
Tank 5 .....	15	1,650	291,000	665,000	298,000	454,000	700,900
Tank 6 .....	14	180,000	525,000	1,660,000	550,000	1,300,000	810,000
Tank 8 .....	15	202,000	360,000	1,244,000	484,000	730,000	900,000
Tank 10 .....	12	290,000	880,000	3,830,000	1,210,000	1,840,000	1,390,000
Average .....	14	162,000	495,000	1,750,000	650,000	1,040,000	930,000
Ratio to Gelatin Count .....	...	9	28	100	35	59	53

SERIES B.

	NO. OF SAMPLES	BACTERIA PER C.C.						
		Microscopic Count	On Lactose Gelatin at 20			Lactose Agar at 37	Nährstoff	Anaërobic Gelatin
			Liquefiers	Acid Formers	Total			
Tank 5..	8	21,525,000	78,000	291,700	926,700	682,500	1,399,400	610,000
Tank 6 ..	5	30,520,000	60,000	126,700	660,000	486,000	1,212,000	306,700
Tank 7 ..	5	42,140,000	37,500	210,000	706,000	456,000	1,271,000	230,000
Tank 8 ..	5	39,620,000	220,000	196,700	1,605,000	738,000	1,338,000	913,300
Tank 9 ..	4	24,425,000	33,300	80,000	34,300	322,500	958,750	163,300
Tank 10 ..	6	22,400,000	47,500	128,000	486,000	346,700	2,888,000	352,000
Average .	5	30,105,000	79,400	172,200	787,800	505,300	1,511,190	429,200
Ratio to Gelatin Count ..	...	3,830	11	22	100	64	192	55

tank ratios with those of the sewage there is manifest in both series a relative decrease of the total on lactose agar at 37° and a very slight increase of anaërobes. The insignificance of the latter is somewhat surprising. A somewhat more marked increase is shown in the ratio of the Nährstoff count; and the ratio of the microscopic count to the gelatin count is doubled, the absolute value of the former being the same as in the case of the sewage.

These facts suggest that the decrease of 20° gelatin forms in passage through the septic tank may be balanced by a multiplication of other bacteria.

#### BACTERIA IN CONTACT FILTER EFFLUENTS.

The analyses of the contact filters, nine in Series A, and seven in Series B, are arranged in Table 11. They show in general that these effluents contained two-thirds as many bacteria as were found in the septic effluents and one-third to one-fifth as many as the crude sewage, or in absolute numbers 500,000 to 1,000,000. At Charlottenburg, Chorley, and Lawrence the numbers have varied from 100,000 to 600,000, while the effluents at Exeter showed one million and at London two to six millions. Smith at Belfast reports over 20 millions. In comparing the individual filters of Table 11 it may be noted that of the double contact beds, the primary pair, 19 and 20, show much higher numbers than the corresponding secondary beds, 17 and 18, 20, which takes septic sewage, being the highest of all.

The various groups of bacteria in contact effluents maintain the same relation as in the crude sewage, with two exceptions.

TABLE 11.

#### BACTERIA IN CONTACT FILTER EFFLUENTS.

##### SERIES A.

	NO. OF SAMPLES	BACTERIA PER C.C.					
		On Lactose Gelatin at 20°			On Lactose Agar at 37°		On Anaërobic Agar
		Liquefiers	Acid Formers	Total	Acid Formers	Total	
Tank 11 .....	17	94,000	262,000	956,000	492,000	624,000	529,000
Tank 12 .....	20	112,000	158,000	1,238,000	387,000	733,000	561,000
Tank 13 .....	18	29,000	76,000	738,000	100,000	215,000	228,000
Tank 14 .....	16	17,000	108,000	776,000	186,000	375,000	338,000
Tank 16 .....	12	66,000	433,000	1,828,000	204,000	361,000	208,000
Tank 17 .....	12	19,000	238,000	496,000	183,000	270,000	281,000
Tank 18 .....	17	43,000	228,000	648,000	239,000	623,000	412,000
Tank 19 .....	12	44,000	368,000	1,038,000	322,000	502,000	388,000
Tank 20 .....	16	105,000	691,000	1,904,000	460,000	1,240,000	950,000
Average .....	15	60,000	270,000	1,060,000	290,000	570,000	440,000
Ratio to Gelatin Count .....	...	6	25	100	27	54	41



TABLE 11—*Continued.*

## SERIES B.

	No. of Samples	BACTERIA PER C.C.						
		Microscopic Count	On Lactose Gelatin at 20°			Lactose Agar at 37°	Nährstoff	Anaërobic Gelatin
			Liquefiers	Acid Formers	Total			
Tank 11..	5	22,600,000	94,200	215,000	689,600	455,000	939,700	510,000
Tank 12..	4	40,875,000	125,000	23,000	897,000	1,045,000	1,263,700	255,000
Tank 13..	5	21,260,000	47,500	93,300	287,500	190,000	1,324,000	250,000
Tank 14..	4	32,525,000	55,000	35,000	665,000	602,500	397,500	935,000
Tank 16..	4	15,827,500	135,000	36,700	240,000	186,700	450,000	257,500
Tank 17..	4	8,625,000	8,100	87,500	185,000	120,000	612,500	80,000
Tank 18..	4	27,225,000	135,000	200,000	682,500	442,500	1,265,000	427,500
Average .	4	24,133,900	85,700	126,800	520,900	434,500	891,800	387,900
Ratio to Gelatin Count . .	...	4,610	16	24	100	83	171	74

The proportion of liquefiers was increased in Series B to 16 per cent of the total on gelatin. In Series A this did not occur, possibly because the beds had not been in operation long enough to exhibit their typical characteristics; but in connection with the results obtained with the trickling filters the figures of Series B suggest that in a loose stone filter there may be an increase in the proportion of liquefying bacteria present. Clowes and Houston (1903), obtained negative results in this regard.

In the second place the ratio of bacteria as determined by the microscopic method showed a very great increase, being nearly fiftyfold that for gelatin; its absolute value was 24,000,000 against 29,000,000 for sewage.

## BACTERIA IN TRICKLING FILTER EFFLUENTS.

The single trickling filter examined in Series A (Table 12), was not in thoroughly satisfactory operation and the analyses of its effluent are somewhat aberrant. Both series show a somewhat smaller total of bacteria than in the contact effluents, about one-quarter of the number present in raw sewage. The ratios of Series B, typical of the beds when in good working order, correspond almost exactly with those of the contact beds, except that they show a higher ratio of liquefiers, 30 per cent of

the total and nearly three times as many bacteria on Nährstoff as on gelatin. Apparently in the filters of these two latter types there is either a multiplication or a relative persistence of certain bacteria which do not grow on gelatin, and of the liquefying forms. Since nitrification and the dissolution of solid materials are among the most important functions of contact and trickling beds these phenomena are significant.

TABLE 12.  
BACTERIA IN TRICKLING FILTER EFFLUENTS.  
SERIES A.

	NO. OF SAMPLES	BACTERIA PER C.C.					
		On Lactose Gelatin at 20°			On Lactose Agar at 37°		On Anaerobic Agar
		Liquefiers	Acid Formers	Total	Acid Formers	Total	
Tank 15....	10	22,000	93,000	1,030,000	260,000	1,010,000	370,000
Ratio to Gelatin Count....		2	7	100	24	94	34

SERIES B.

	NO. OF SAMPLES	BACTERIA PER C.C.						
		Microscopic Count	On Lactose Gelatin at 20°			Lactose Agar at 37°	Nährstoff	Anaerobic Gelatin
			Liquefiers	Acid Formers	Total			
Tank 15 ....	8	23,633,000	127,500	98,800	258,700	410,000	1,228,500	228,700
" 22 ....	5	20,080,000	192,000	120,000	678,000	676,000	1,739,000	370,000
" 23 ....	5	9,425,000	20,600	124,200	415,000	66,500	557,000	240,000
Average ....		17,729,000	133,700	114,100	450,600	284,200	1,174,800	279,600
Ratio to Gelatin .....		3,940	30	25	100	63	260	62

BACTERIA IN SAND FILTER EFFLUENTS.

The bacterial analyses of sand filter effluents in Table 13 correspond with those obtained at Chorley, Oxford, Lawrence, Worcester, and Ames in showing numbers varying from 1,000 to 50,000 per c.c. The West Derby and Exeter figures are somewhat higher.

TABLE 13.  
BACTERIA IN SAND FILTER EFFLUENTS.  
SERIES A.

	NO. SAMPLES	BACTERIA PER C.C.					
		On Lactose Gelatin at 20°			On Lactose Agar at 37°		On Anaërobic Agar
		Liquefiers	Acid Formers	Total	Acid Formers	Total	
Tank 1.....	14	120	470	1,220	330	630	320
Ratio to Gelatin Count.....		10	38	100	26	50	26

SERIES B.

	NO. SAMPLES	BACTERIA PER C.C.						
		Microscopic Count	On Lactose Gelatin at 20°			Lactose Agar at 37°	Nähr- stoff	Anaë- robic Gela- tin
			Lique- fiers	Acid Formers	Total			
Tank 1.....	5	1,320,000	155	400	7,900	17,000	66,000	770
" 24.....	5	340,000	680	2,600	15,600	14,400	44,000	700
" 25.....	5	300,000	670	1,100	3,970	2,900	21,000	2,160
Average.....		650,000	500	1,360	9,160	11,400	43,600	1,210
Ratio to Gelatin Count.....		7,100	5	15	100	120	480	13

With regard to ratios on different media, three points are noticeable. The proportion of anaërobes in each series is distinctly lower than in any other type of effluent, being only half that of the sewage in Series A and less than a third in Series B. The ratio of the Nährstoff count and the microscopic count on the other hand is higher for the sand filter effluents than for any others, three times that of sewage for the Nährstoff count and four times that of sewage for the microscopic count.

#### CONCLUSIONS.

The results of this preliminary study may be briefly summarized as follows:

1. The day flow of Boston sewage contains on an average from one to five million bacteria per c.c. as determined by plating on

TABLE 14.

RATIOS OF COUNTS BY MICROSCOPIC METHOD AND BY PLATING ON VARIOUS MEDIA TO THE COUNT ON LACTOSE GELATIN AT 20°. SEWAGES AND EFFLUENTS.

## SERIES A.

SOURCE OF SAMPLE	AVERAGE NO. OF BACTERIA PER C.C. LACTOSE GELATIN AT 20°	RATIO OF NO. OF BACTERIA TO THE NO. ON LACTOSE GELATIN						At 20°		
		Microscopic Count	On Lactose Gela- tin at 20°			On Lactose Agar at 37°		On Anaërobic Gelatin	On Anaërobic Agar	On Nährstoff
			Liquefiers	Acid Formers	Total	Acid Formers	Total			
Sewage .....	5,430,000		7	31	100	31	69		45	
Septic Tanks .....	1,750,000		9	28	100	35	59		53	
Contact Filters...	1,060,000		6	25	100	27	54		41	
Trickling Filters.	1,030,000		2	7	100	24	94		34	
Sand Filters.....	1,250		10	38	100	26	50		26	

## SERIES B.

Sewage .....	1,690,000	1,700	9	25	100		83	50		170
Septic Tanks .....	787,000	3,830	11	22	100		64	55		192
Contact Filters...	520,900	4,610	16	24	100		83	74		171
Trickling Filters.	450,600	3,940	30	25	100		63	62		260
Sand Filters.....	9,160	7,100	5	15	100		120	13		480

lactose gelatin at 20°. Of these 7 to 9 per cent are liquefiers and 25 to 30 per cent acid formers.

2. Lactose agar counts at 37° show 70 to 80 per cent as many colonies as on gelatin at 20° with the same absolute number of acid formers as at the lower temperature.

3. Anaërobic cultures show about one-half as many colonies as corresponding cultures under aërobic conditions.

4. Nährstoff agar gives counts not quite twice as high as those obtained by the use of lactose gelatin.

5. Direct microscopic enumeration shows nearly 20 times as many bacteria as appear upon gelatin plates.

6. The bacteria in Boston sewage exhibit a marked seasonal variation with a maximum in September and a minimum during the winter months.



7. In passage through the septic tank the number of bacteria on gelatin falls off one-half, while 37° counts show a slightly less decrease. The microscopic count remains unchanged.

8. In passing through contact and trickling filters the number of bacteria on gelatin is reduced to one-third of its sewage value, or less. The proportion of liquefiers is doubled or trebled. The microscopic count is only from one- to two-fifths less than in the case of sewage.

9. Sand filter effluents contain about one-half of one per cent as many bacteria as raw sewage on the gelatin plate, over one per cent measured on Nährstoff agar and about two per cent as shown by the microscopic count. The anaërobes are more markedly decreased than the aërobes.

10. The count upon the gelatin plate, in sewage purification as elsewhere, appears to correspond well with the amount of decomposable organic matter. The number of bacteria as determined in this way, decreases with the amount of purification effected and may furnish an indirect measure of it.

11. The total number of bacteria as determined by direct microscopical enumeration does not decrease directly with the gelatin count. Its ratio to the latter is highest in the purest effluents. In view of the fact that the nitrifying bacteria do not appear on the gelatin plate this result is somewhat significant.

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# THE MODE OF ACTION OF THE CONTACT FILTER IN SEWAGE PURIFICATION.

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## INTRODUCTION.

THE contact system of sewage purification is now so well known that only a brief description of the process will be necessary in this paper. It consists in the use of a bed of coarse stone, coke, or other hard material, of from one-eighth inch to two inches in diameter; the bed is usually five or six feet deep, although greater depths have been used; is filled completely with sewage and allowed to stand full for a given period, usually two hours; it is then allowed to drain slowly and it stands empty for six or eight hours or longer.

This contact filter, or bacterial bed, as it was called at first, was introduced by Dibdin in the course of a series of experiments carried out by him for the London County Council on the sewage of London. The first of these experiments were made upon sand filters (intermittent downward filtration), and the contact filter was gradually evolved from this earlier type. The first contact filters were run continuously 16 hours out of 24, the outlet being so regulated that the filter-bed stood full during this period. It was then emptied and rested empty for a period of eight hours. Later these London beds were operated with a shorter full period as are all contact beds today.

## THEORIES OF THE ACTION OF THE CONTACT BED.

Dibdin (1897),<sup>1</sup> in his earlier writings at least, seems to have been influenced in his conception of the action of the contact bed by the fact that, in his experiments, it was developed from the sand filter. He accordingly offers no special explanation of the manner in which the contact bed purifies sewage, assuming the action to be the same in the two types of filter. That such is the case we strongly doubt, since, it has never been shown that in a sand filter there is any extensive alternation of oxidizing and reducing reactions such as characterizes the working of the contact bed. In his testimony before the Royal Commission on Sewage Disposal, Dibdin (1902)<sup>2</sup> made the following statement concerning the action of the contact bed:

During that period of two hours rest, . . . each cavity becomes really a settling chamber. The solid matter in the sewage by the action of gravity, subsides or adheres to the larger particles as chips of wood in water will to a boat, and when you draw the water away from the bed these solid matters are left behind and the bacteria gradually extend over them, and in the course of time they become solutionized . . .

This view is certainly correct as far as it goes. It must be remembered, however, that half the organic matter of the sewage is already in a soluble state and will not be deposited in any such manner, and also that purification seems to be easier in the case of matter in solution than in that of suspended matter. That such is the case will be evident from the fact that purification goes on more rapidly in the case of clarified sewage.

Dunbar and Thumm (1902)<sup>3</sup> carried out at the Hamburg Experiment Station an extensive study of the contact bed; and their conclusions in brief are as follows: The agencies of the contact bed are three in number: mechanical straining, physical absorption, and biological activity. They place the greatest emphasis upon the absorption phenomena which they demonstrate by well planned experiments but which they do not attempt to explain. Absorption, according to these investigators, is dependent upon (1) bacterial activity, and (2) aëration, and may be

<sup>1</sup> *The Purification of Sewage and Water*, London, 1897, p. 48.

<sup>2</sup> *Interim Report of the Royal Commission on Sewage Disposal*, London, 1902, 2, p. 232, Quest. 3905.

<sup>3</sup> *Beitrag zum derzeitigen Stande der Abwässerreinigungsfrage, mit besonderer Berücksichtigung der biologischen Reinigungsverfahren*, Berlin, 1902.



checked by the removal of either of these factors. They are careful to state, however, that the action of the bacteria is simply to remove the absorbed material and thus prevent a saturation of the absorbing agent. The necessity of aëration is a similar phenomenon, the oxygen being necessary for the life of the bacteria in question. By the term "oxidation process" (*oxydationsverfahren*) which they apply to the contact system, it is not intended to indicate that the products of oxidation, such as nitrates, must be present in the effluent, but rather that oxygen is applied to the decomposable material at such an early stage and in such an amount that offensive putrefaction is prevented. The formation of nitrates and nitrites is, in the opinion of these authors, of secondary importance. Nitrates are formed only in the empty period and, in the author's belief, not exclusively by the Winogradsky organisms. Nitrites, in general, result from the reduction of the nitrates during the full period. No attempt is made to follow the course of these important compounds further.

Harry W. Clark (1902),<sup>1</sup> chemist of the Massachusetts State Board of Health, as a result of experiments carried out at the Lawrence Experiment Station, agrees with Dunbar that nitrification occurs more actively when the filter is standing empty, but he also adds that "nitrification certainly occurs when a filter is filled with sewage until such a time as the air is exhausted," and cites an experiment in which higher nitrates were found in the effluent at the end of the discharge than at its beginning. This must mean not only that the formation of nitrates is taking place during the full period but that it is taking place at a more rapid rate than is their reduction, there being an actual increase in the nitrates in the sewage. Clark further states (1903),<sup>2</sup> "that it has not been the general experience at Lawrence that good purification can be obtained without nitrification," and believes that nitrates are essential in a satisfactory effluent. His views are thus in so far opposed to those of Dunbar and Thumm.

The points at issue are not immaterial as might at first seem to be the case, but are of fundamental importance to the proper control of the process. Our chemical data show us the initial and

<sup>1</sup> *Annual Report of the Massachusetts State Board of Health for 1901, 1902*, p. 276.

<sup>2</sup> *Annual Report of the Massachusetts State Board of Health for 1902, 1903*, pp. 198, 203.

final states of the sewage as it enters and leaves the filter, but we have hitherto been in the dark as to the nature of the process itself. The purification of sewage has been likened to a combustion or burning up of organic matter and, judged by the end products alone, the reactions are not dissimilar. We know, however, that the process of oxidation which goes on in a filter is a much more complex phenomenon than any direct combustion. Many intermediate products are known in the former case which are unknown in the latter, and a closer study of the end products shows that they also differ considerably.

In the hope of throwing light upon the details and intermediate stages of this process and of determining the mode of action or, so to speak, the physiology of the contact bed, we have made an extensive series of chemical analyses of the contents of a working contact sewage filter, drawn from various levels of the filter and during various phases of its contact cycle with results which we believe to be of theoretical interest and practical importance.

#### TERMINOLOGY.

The term "bacterial bed" which was originally applied to the contact filter was inappropriate since all of the so-called biological filters are also bacterial filters. The term "contact filter" seems most fortunate emphasizing as it does the physical and chemical phenomena in the action of the filter.

The operation of the filter is divided into two periods; first, the period of filling and standing full, commonly and very naturally spoken of as the full period, and second, the period of emptying and standing "empty," often called the resting period. This term "resting period" seems to us to be peculiarly inappropriate, since as has previously been pointed out and as we shall have occasion to show in this paper, there is taking place at this time within the filter the most essential reaction of the whole process, namely, the oxidation of the nitrogenous and carbonaceous matter of the sewage. Moreover, while the term "full period" is not inappropriate, it at least is expressive only of the physical condition of the tank and gives no indication of the occurrence or nature of the important reactions which take place during that period.

In view of these facts we have deemed it advisable to apply to the various stages of the cycle a set of terms which shall be not only accurate as to fact but in so far as possible suggestive of the processes taking place during these stages. For the first stage, that of filling, we use the term "filling phase." The next period in which the filter stands full we have called the "reduction phase." The word reduction in this connection has a double significance, since as we propose to show, there are taking place during this period two strictly chemical reductions,—of the nitrates and of the nitrites,—and further, that in the broad sense the whole mass of sewage is being reduced, digested, or broken down into simpler forms both through the agency of the bacteria and through strictly chemical reactions.

For the third period of the cycle we prefer the term "emptying phase" to the more commonly used "draining period." Draining suggests the slow running off of the last portions of the sewage rather than the more rapid discharge of the whole dose and if used at all would be more appropriately applied to the latter stages of the emptying. Finally, for the last or empty period there seems to be no more appropriate term than "oxidation phase." This stage is the oxidation period *par excellence*, although there is also a certain amount of oxidation going on during the full or reduction phase. These terms, having been of service to us in our work, are therefore recommended for general adoption in the interest of both accuracy and uniformity.

#### PLAN OF THE WORK.

The study naturally divides itself into two parts corresponding to the two general periods in the operation of the filter. First, the filling and reduction phases, as already defined, and second, the emptying and oxidation phases. The study of the latter phase will naturally consist in analyses of the gas contained in the tank and of certain changes which take place on the filtering material, while in the former case it will be necessary to study the changes taking place in the sewage itself. In carrying out the work we have made use of the "oxygen cycle," instead of the usual "nitrogen cycle," attempting to follow the history of the

oxygen in the process and in this way to trace out the reactions of the successive phases.

Our experiments were carried out at the Sewage Experiment Station with filter Number 16, which is shown in the accompanying plate. This filter consists of a cypress tank, four feet square in area and six feet deep, filled with crushed trap-rock and granite of such a size that it would pass through a one-half inch mesh and be retained on a one-fourth inch mesh. The tank is provided with five pet-cocks, one foot apart, the first one being one foot below the surface of the stone. The pet-cocks, together with the effluent cock, provide means for sampling at six levels either the contained sewage during the reduction phase or the gases during the oxidation phase. The tank was first put in operation in June, 1903, and has been receiving three fillings daily of raw sewage at eight hour intervals for six days in the week. The method of operation has been to fill the tank in one hour, allow it to stand full for two hours, empty in one hour and allow a period of four hours before the next filling. The filter was out of service for one month in February, 1904. The averages of 78 analyses of the raw sewage and of 16 analyses of the effluent of this filter is given below. These analyses were made during the six months previous to the commencement of this work.

## PARTS PER MILLION.

SAMPLE	TEMP.	NITROGEN AS				OXYGEN CONSUMED	OXYGEN DIS- SOLVED
		Free Ammonia	Albumi- noid Ammonia	Nitrites	Nitrates		
Sewage	62.0	21.3	6.89	0.30	0.08	50.0	3.7
	61.2	14.4	2.32	0.99	0.00	17.4	0.7

## METHODS OF ANALYSIS.

*Analyses of the gas.*—The gas analyses were made on a regular, portable, Orsat apparatus, as described by Gill (1897).<sup>1</sup>\*

*Dissolved oxygen.*—The determination of the dissolved oxygen was made according to the procedure of Winkler (1888).<sup>2</sup>

<sup>1</sup> *Gas and Fuel Analysis for Engineers*, New York, 1897, p. 11.

\*The apparatus for this work was kindly loaned to us by Professor A. H. Gill, to whom the authors desire to express their thanks.

<sup>2</sup> *Berichte d. Deutsche Chem. Gesellsch.*, 1883, 21, p. 2843.





TANK 16 (Center Background)

*Nitrates*.—The determination of the nitrates was made by the Brucine method of Noll (1901),<sup>1</sup> as described by Farnsteiner and his associates (1902).<sup>2</sup>

*Nitrites*.—Nitrites were determined by the Griess method as described by Richards and Woodman (1904).<sup>3</sup>

Although the two general periods in the operation of the filter were studied successively throughout this work, it will aid in a clearer understanding of the results to present them separately.

#### OXIDATION PHASE.

Our work upon the oxidation phase consisted in studying the gas contained within the filter, the changes taking place in this gas during the phase and the difference in the composition of the gas at different depths within the filter. We also studied the chemical changes taking place upon the surface of the filtering material, and carried out some experiments with special apparatus

TABLE 1.  
ANALYSES OF THE GAS FROM THE MIDDLE PET-COCK OF FILTER 16, TAKEN AT THE END OF THE FOUR HOUR OXIDATION PHASE.

DATE—1904	TEMP. C.	PERCENTAGES		
		Carbon Dioxide	Oxygen	Nitrogen (By difference)
March 10.....	47	0.4	18.5	81.1
“ 10.....	47	0.6	17.6	81.8
“ 16.....	49	0.4	18.8	80.8
“ 17.....	49	0.4	18.4	81.2
“ 18.....	48	0.4	18.8	80.8
“ 21.....	48	0.9	15.4	83.7
“ 23.....	48	1.3	16.4	81.9
“ 24.....	48	0.9	16.4	84.5
“ 26.....	53	0.8	17.7	81.5
“ 28.....	49	0.8	14.5	84.6
“ 29.....	50	1.5	16.7	81.8
April 2.....	49	0.8	16.0	83.2
“ 6.....	51	2.2	14.0	83.8
“ 8.....	51	1.5	17.5	81.0
May 9.....	56	2.2	17.5	80.3
Average.....	..	1.0	16.9	82.1
Air of the Filter House.	..	0.0	20.8	79.2

<sup>1</sup> *Ztschr. f. ang. Chemic*, 1901, 14, p. 1317.

<sup>2</sup> *Leitfaden für die Chemische Untersuchung von Abwässer*, Hamburg, 1902.

<sup>3</sup> *Air, Water and Food*, New York, 1930, p. 94.

designed to test certain of the points brought out by our study. In Table 1 are given the analytical results of fifteen sets of gas analyses, together with an analysis of the normal air of the filter-house. The analyses were made at the conclusion of the four-hour oxidation phase and serve to illustrate the change taking place in the gas within the tank during that phase. The samples were in each case taken from the middle pet-cock.

A study of these figures shows clearly, first, that oxygen is absorbed or in some way used up in the filter and, second, that the whole of the oxygen thus taken up does not reappear as carbon dioxide, a portion of it disappearing entirely.

In order further to study the absorption of the oxygen by the filtering material we made the following experiments:

#### EXPERIMENT 1.

A bottle of about two liters capacity was filled with some material taken from the top of filter No. 16. This bottle was filled with sewage, allowed to stand for two hours, and then slowly emptied by means of a siphon. Air was immediately passed through it at a rate of about two liters per hour. Analyses of the air were made before and after passing through the bottle. The following results were obtained:

Sample	Carbon Dioxide	Oxygen	Nitrogen
Before passing - - - -	0.0	20.8	79.2
After passing - - - -	1.4	18.7	79.9

This experiment confirmed, as was anticipated, the results of Table 1, and shows that the absorption at the beginning of the oxidation phase takes place at a somewhat rapid rate.

#### EXPERIMENT 2.

To study those changes in the gases in a strictly quantitative manner, we devised a second experiment as follows:

The apparatus shown in Fig. 1 was used. *A* is a bottle filled with material taken from the top of filter No. 16. It was first filled with sewage and allowed to stand two hours; then the sewage was siphoned off and air admitted at *a*. The volume of the sewage removed, and thus of the air admitted, was measured. At the time of filling the bottle with sewage the whole apparatus was immersed in a pail of the same sewage in order to maintain as even a temperature as possible and especially to prevent sudden fluctuations.

The cylinder, *B*, was then placed in position and filled up to a definite mark with water, *a* being closed.

As oxygen was absorbed from the gas within the bottle, water was drawn in from *B*, and in order to make a reading at any time it was only necessary to add water to *B* from a graduate, filling it up to the original mark, and note the amount of water required. Temperature readings were made at the same time, and the proper corrections applied to the readings. Two series of observations were made with this apparatus, the results of which are given in Table 2. In each instance an analysis of the residual gas was made.

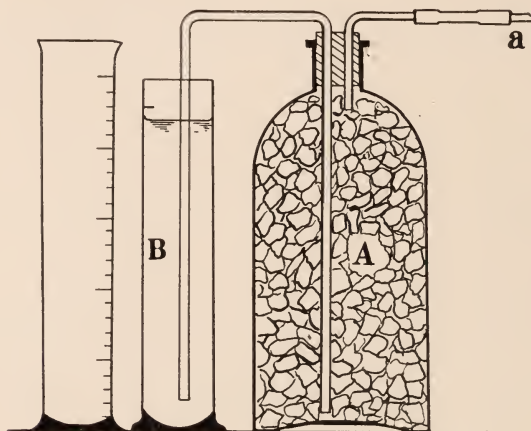


FIG. 1.

TABLE 2.

TABLE SHOWING APPROXIMATELY THE RATE OF ABSORPTION OF OXYGEN BY THE FILTERING MATERIAL OF A CONTACT FILTER.

DATE	HOUR	TEMPERATURE	LOSS IN VOLUME		ANALYSIS OF RESIDUAL GAS	
			Measured	Corrected	CO <sub>2</sub>	O <sub>2</sub>
May 20.....	4 P. M.	15.0	Start			
	10 P. M.	15.3	29 c.c.	30 c.c.		
21.....	4 A. M.	14.0	53 c.c.	49 c.c.	6.0	3.8
	8 A. M.	14.0	64 c.c.	60 c.c.		
22.....	8 A. M.	17.0	93 c.c.	102 c.c.		
May 23.....	4 P. M.	13.0	Start			
	5 P. M.	13.0	12.5 c.c.	12.5 c.c.		
24.....	11 A. M.	11.0	107.5 c.c.	100.5 c.c.	7.3	0.0
25.....	11 A. M.	15.0	122.5 c.c.	129.0 c.c.		

Original volume in first series, 1,000 c.c., in second, 1,030 c.c.

Obviously if there are no other gases produced during the experiment, the total loss in volume will be the loss in oxygen minus the production of



carbon dioxide. Arranging our results from this point of view, we obtain the figures given in

TABLE 3.

	I	II
1. Original volume of air - - - - -	1,000 c.c.	1,030 c.c.
2. Loss in volume (observed) - - - - -	102 c.c.	129 c.c.
3. Per cent loss (observed) - - - - -	10.2%	12.5%
4. Oxygen absorbed - - - - -	17.0%	20.8%
5. Carbon dioxide evolved - - - - -	6.0%	7.3%
6. Loss in volume (calculated) - - - - -	11.0%	13.5%
7. Calculated loss (6) minus observed loss (3) - - -	0.9%	1.0%

It appears from these two sets of observations that about one-third of the total volume of oxygen used up within the filter during the oxidation phase reappears as carbon dioxide, and that the remainder is held on the surface of the filtering material in some fixed form, probably as nitrates. In addition to this loss of oxygen there is the production of an inert gas to the extent of about one per cent of the total volume. This gas is probably nitrogen, as we shall show in our discussion of the reduction phase.

A few sets of analyses were made at the beginning of the oxidation phase of the gases at the different levels, and the differences observed were either very slight or altogether wanting. There is practically no variation in the composition of the gas throughout the tank at this stage.

In order to study the chemical changes taking place on the surface of the filtering material, we made the following experiment:

We prepared six wooden boxes, four inches square and four inches deep inside, with perforated bottoms and open tops. These boxes were buried in the material of filter No. 16 and were then filled with the material displaced. The tops of the boxes were flush with the surface of the filtering material. They thus constituted portions of the tank itself which could be removed at any time for examination. Three days later, and immediately after drawing down the sewage at the end of one of the two-hour reduction phases, one of these boxes was removed and its contents used to fill a wire basket of about one liter capacity. This basket was then washed by pouring one liter of water through it four times, and the wash water thus obtained was examined for nitrates and nitrites. Other boxes were removed later, at intervals, and similarly examined. In this way it was possible to follow the nitrate and nitrite production in the filter during the oxidation phase without disturbing the main body of the filter. The results obtained in these experiments are given in Table 4.

TABLE 4.  
FORMATION OF NITRATES AND NITRITES ON THE SURFACE OF THE  
FILTERING MATERIAL OF A CONTACT FILTER DURING  
THE OXIDATION PERIOD.

TIME	NITROGEN AS	
	Nitrates	Nitrites
11:30 A. M. ....	0.0003 mg.	0.0006 mg.
12:00 M. ....	0.0009 mg.	0.0001 mg.
I. 1:00 P. M. ....	0.003 mg.	0.0001 mg.
4:00 P. M. ....	0.004 mg.	0.0001 mg.
II. 4:00 P. M. ....	0.009 mg.	0.0000 mg.
III. 4:00 P. M. ....	0.007 mg.	0.0001 mg.

Outlet cock opened and tank started to empty in each case at 11:30.

The first four determinations constitute one series, and show in a general way the formation of nitrates and the loss of nitrites during the oxidation phase. The two single sets were made on two other days at the end of the four-hour oxidation phase.

The significance of the figures lies in their relation to the free oxygen in the bed. It was seen in Experiments 1 and 2, and in the analyses of the tank gases given in Table I, that a large amount of oxygen was used up in the bed during this phase which did not reappear as carbon dioxide. A portion of that oxygen is evidently used up in the production of nitrates.

In addition to these experiments some data on this same point may be gathered from the analyses of the sewage within the tank during the filling period. During a portion of the time the night filling of this tank was omitted and it had therefore a twelve hour oxidation phase alternating with one of four hours. On filling with sewage and examining the first sewage passing through the bed of the filter the nitrates were found to be much higher after the longer period of standing empty. For example:

Time	After Empty Period of	Nitrogen as Nitrate Parts per Million
April 22, 4 P. M. ....	4 hours	1.11
April 23, 8 A. M. ....	12 "	5.00

These figures also show that the production of nitrates goes on rapidly during the empty period, and that a longer period of rest gives a corresponding amount of oxidized nitrogen.

In general the reactions of the oxidation phase appear to be as follows. The organic matter within the filter-bed is oxidized at the expense of the free oxygen. A part of the carbon is converted into carbon dioxide and escapes in the gaseous form. Some of the nitrogen is converted into the higher oxides, nitrogen trioxide and nitrogen pentoxide. All these reactions taken together, however, do not account for more than two-tenths of the oxygen used up. A large portion of this gas must go toward the partial oxidation of the organic matter, producing compounds which contain sufficient oxygen to render them stable and unobjectionable in an effluent, but which are, nevertheless, capable of further oxidation. Such bodies we know do exist in perfectly satisfactory effluents, in which the "albuminoid ammonia," and "oxygen consumed" values may be quite high, but which, if submitted to the incubation test, are found to be non-putrescible.

#### THE REDUCTION PHASE.

Our studies of this phase consisted in analyses of the sewage as it flowed in, samples being taken from the various pet-cocks, and also of the sewage at various intervals during the period, to ascertain what changes were taking place within the filter. Determinations of the four conditions of the oxygen, namely the free and dissolved oxygen, and the oxygen as carbon dioxide, nitrates and nitrites were made. The analyses given in Table V, under the heading "initial," were obtained by allowing the sewage to flow into the tank as usual and, starting with the lower pet-cock, taking a sample of the sewage from each pet-cock, as it reached that point. In this way the samples represented successive washings of the stones above. The pet-cocks are lettered in color, A being at the top. At the end of the two-hour period similar samples were taken. The results are given in Table 5 under the heading "final."

This table shows in a general way that, in the action of the contact filter, the dissolved oxygen and the oxygen of the nitrates

TABLE 5.

ANALYSES OF THE APPLIED SEWAGE, AND OF THE SEWAGE WITHIN THE BED OF A CONTA  
 FILTER, TAKEN FROM FIVE LEVELS AT THE BEGINNING AND THE END OF THE REDU  
 TION PERIOD.

(Parts per Million.)

DATE TEMP. F.	PET-CKCK	DISSOLVED OXYGEN		NITROGEN AS			
				NITRATES		NITRITES	
		Initial	Final	Initial	Final	Initial	Final
March 25 44°	Applied sewage	7.8	...	1.0	...	0.02	....
	A	7.5	0.0	0.9	5.6	0.05	0.12
	B	8.5	0.0	1.0	1.2	0.05	0.60
	C	8.9	0.5	0.9	1.7	0.06	0.80
	D	9.5	0.0	1.3	2.3	0.06	0.05
	E	9.7	0.0	3.0	1.7	0.06	0.09
	Average	8.7	0.1	1.4	2.5	0.05	0.33
March 28 44°	Applied sewage	7.8	...	0.30	....	0.06	....
	A	5.9	0.7	1.30	0.50	0.09	0.40
	B	8.3	1.4	1.80	1.10	0.09	0.70
	C	8.1	2.8	1.30	0.85	0.10	1.00
	D	6.8	0.9	1.30	1.00	0.06	0.58
	E	9.3	0.2	2.15	1.45	0.08	0.22
	Average	7.7	1.2	1.4	1.00	0.08	0.58
March 30 51°	Applied sewage	9.1	...	0.40	....	0.132	.....
	A	5.5	1.9	1.35	0.90	0.444	0.500
	B	6.0	0.4	1.85	0.50	0.444	0.400
	C	6.4	0.2	0.85	0.00	0.284	0.150
	D	6.4	0.1	1.10	0.00	0.200	0.050
	E	7.5	1.1	2.25	0.40	0.136	0.065
	Average	6.8	0.7	1.30	0.36	0.273	0.233
March 31 51°	Applied sewage	9.4	...	0.30	....	0.160	....
	A	4.6	0.0	0.80	0.10	0.340	0.10
	B	5.5	0.5	0.60	0.15	0.300	0.40
	C	6.3	2.1	0.60	0.40	0.240	0.70
	D	5.5	1.6	0.65	0.40	0.260	0.65
	E	9.5	0.8	1.65	1.10	0.200	0.40
	Average	6.8	1.0	0.60	0.43	0.250	0.45
April 1 49°	Applied sewage	9.1	...	0.14	....	0.168	....
	A	5.0	0.0	0.20	0.03	0.276	0.16
	B	5.5	0.0	0.18	0.02	0.200	0.04
	C	5.9	0.0	0.16	0.02	0.200	0.00
	D	7.1	0.1	0.18	0.01	0.234	0.02
	E	7.7	0.4	0.40	0.22	0.200	0.20
	Average	6.7	0.1	0.21	0.06	0.213	0.08
April 12 51°	Applied sewage	4.4	...	0.13	....	.....	....
	A	2.3	0.0	0.35	0.16	.....	....
	B	3.8	0.2	0.34	0.02	.....	....
	C	4.8	0.6	0.45	0.30	.....	....
	D	5.0	0.4	0.80	0.60	.....	....
	E	6.0	0.1	1.17	0.71	.....	....
	Average	4.4	0.3	0.54	0.36	.....	....



TABLE 5—Continued.

(Parts per Million.)

DATE TEMP. F.	PET-CK	DISSOLVED OXYGEN		NITROGEN AS			
				NITRATES		NITRITES	
		Initial	Final	Initial	Final	Initial	Final
April 18 53°	Applied sewage	3.5	...	0.06	....	0.052	.....
	A	2.3	0.0	0.32	0.00	0.104	0.000
	B	3.4	0.0	0.32	0.00	0.112	0.000
	C	3.2	0.2	0.70	0.02	0.108	0.400
	D	4.0	0.0	0.80	0.49	0.118	0.120
	E	5.8	0.0	2.30	1.00	0.106	0.000
	Average	3.7	0.0	0.90	0.30	0.100	0.104
April 20 52°	Applied sewage	5.6	...	0.10	....	0.064	.....
	A	1.6	0.4	0.39	0.14	0.228	0.500
	B	4.7	...	0.39	0.03	0.258	0.200
	C	5.4	0.0	0.78	0.18	0.200	0.390
	D	6.7	0.0	1.00	0.59	0.140	0.195
	E	5.4	0.0	1.11	0.72	0.104	0.000
	Average	4.9	0.1	0.63	0.33	0.166	0.257
April 22 53°	Applied sewage	4.1	...	0.11	....	0.263	.....
	A	1.1	0.0	1.72	0.10	0.370	0.252
	B	1.7	0.6	2.94	0.40	0.418	0.312
	C	2.2	0.3	3.34	1.32	0.434	0.498
	D	2.7	0.3	3.57	1.36	0.400	0.334
	E	3.1	0.4	5.00	1.79	0.400	0.338
	Average	2.5	0.3	2.78	1.00	0.381	0.347
Averages 51°	Applied sewage	6.76	....	0.282	.....	0.115	.....
	A	3.98	0.33	0.803	0.837	0.238	0.254
	B	5.22	0.40	1.047	0.380	0.234	0.332
	C	5.68	0.74	1.009	0.532	0.203	0.492
	D	5.96	0.38	1.190	0.751	0.184	0.250
	E	7.11	0.33	2.114	1.010	0.161	0.164
	General average	5.78	0.40	1.070	0.702	0.189	0.298

are used up during the reduction phase, the latter being reduced to about 70 per cent of its original value and the former to about seven per cent. The nitrites show an increase of about 50 per cent. A study of the initial values at the various levels, however, makes it appear probable that a much more complex reaction is taking place than would be indicated by a mere statement of the final results. It will be noticed that the initial samples from the bottom pet-cock *E* show in each case the largest amount of dissolved oxygen and also of nitrates. This is what might be expected since the latter are being washed from the filtering

material and the former is being absorbed from the air with which the tank is filled, as the sewage passes in thin films over the stones of the filter. One would also expect to find a similar relation in respect to the nitrite value. As a matter of fact, however, the largest amount of nitrite is found either at one of the middle pet-cocks or even, in one case, at the top. Since nitrites are very soluble the only explanation that we are able to offer of this important fact is that the nitrites are rapidly changed by reaction with the incoming sewage and, during the time that it takes the sewage to trickle over the stones to the lower levels, this reaction causes a very noticeable decrease in the amount of nitrites present.

It is of special importance to note the extreme reactivity of the nitrites in this process as compared with the much more stable behavior of the nitrates. In our conclusions we shall take up this reaction again and call attention to its great importance in the action of the filter.

TABLE 6.  
ANALYSES OF THE SEWAGE FROM THE BOTTOM PET-COCKS AT  
THE TIME OF FILLING AND 20-MINUTE  
INTERVALS THEREAFTER.  
(Parts per Million.)

TIME	DISSOLVED OXYGEN	NITROGEN AS	
		Nitrates	Nitrites
April 7,			
8:00 A. M.	3.13	1.28	0.74
8:20	...	1.66	0.77
8:40	2.73	2.00	1.54
9:00	2.55	2.27	0.98
9:20	1.95	2.22	1.29
9:40	1.70	2.00	1.00
April 25,			
8:00 A. M.	1.92	1.57	0.43
8:20	0.75	1.93	0.29
8:40	0.54	1.52	0.27
9:00	0.26	1.43	0.24
9:20	0.11	1.25	0.16
9:40	0.00	1.19	0.10

In connection with the reduction phase we also made two series of examinations to show the progressive changes taking place during this period. For this purpose samples were drawn

from the lower pet-cock at the time of filling and at intervals of 20 minutes from that time to the end of the period. The results of these tests are given in Table 6. Unfortunately on the days selected for these examinations the nitrates in the tank were considerably above the average. The figures are of interest, however, as showing the increase in both the nitrates and nitrites during the first portion of the period and then their falling off. Where these figures have been used in connection with others in the general diagram of the cycle, the average of these two series has been reduced by a constant factor to bring them into agreement with the average of the nine series of tests given in Table 5.

*Rate of Discharge of the Filter.*—It was of importance in our calculations to know the rate of discharge of the tank throughout the whole period of this discharge. For this purpose we ran the effluent of the tank into our large measuring tank and made readings of the guage in this tank at short intervals. The results are shown in Table 7 and will be given later in graphical form.

TABLE 7.  
RATE OF DISCHARGE OF THE FILTER-TANK.

ELAPSED TIME, MINUTES	QUANTITY PASSED IN	
	U. S. Gallons	Per cent of Total
5.....	24	10.9
10.....	48	21.8
15.....	71	32.3
20.....	93	42.3
25.....	114	51.8
30.....	134	60.9
35.....	152	69.1
40.....	168	76.4
45.....	181	82.3
50.....	191	86.8
60.....	208	94.5
75.....	214	97.3
110.....	215	97.7
260.....	220	100.0

## SUMMARY OF THE ANALYTICAL DATA. GENERAL CONCLUSIONS CONCERNING THE MODE OF ACTION OF THE CONTACT BED.

Having now presented in detail the analytical data obtained in our studies of the various phases in the operation of the contact filter, we will attempt to summarize the figures so obtained and to construct from them the complete cycle of this filter. Obviously such a cycle will be ideal and only roughly quantitative. In the actual work of the filter the successive cycles of operation differ from one another to a greater or less degree. Many variables combine to produce this difference, chief of which is the composition of the sewage itself. We are too prone to regard sewage as having a definite and fixed composition, while we know, as a matter of fact, that it varies within fairly wide limits, both as to the amount of the organic matter present and as to the amount and nature of the putrifiable changes which have taken place within it. A second and hardly less important variable to be considered is temperature, which affects directly the biological and chemical activity of the filter-bed, and which also determines to a considerable degree the composition of the sewage. More important than either of these factors, however, seems to be the condition of the filter-bed itself. When such a bed is in good working order it behaves like a delicate organism, is most sensitive to any sudden changes in the environment and exhibits its good days and poor days just as a living organism does.

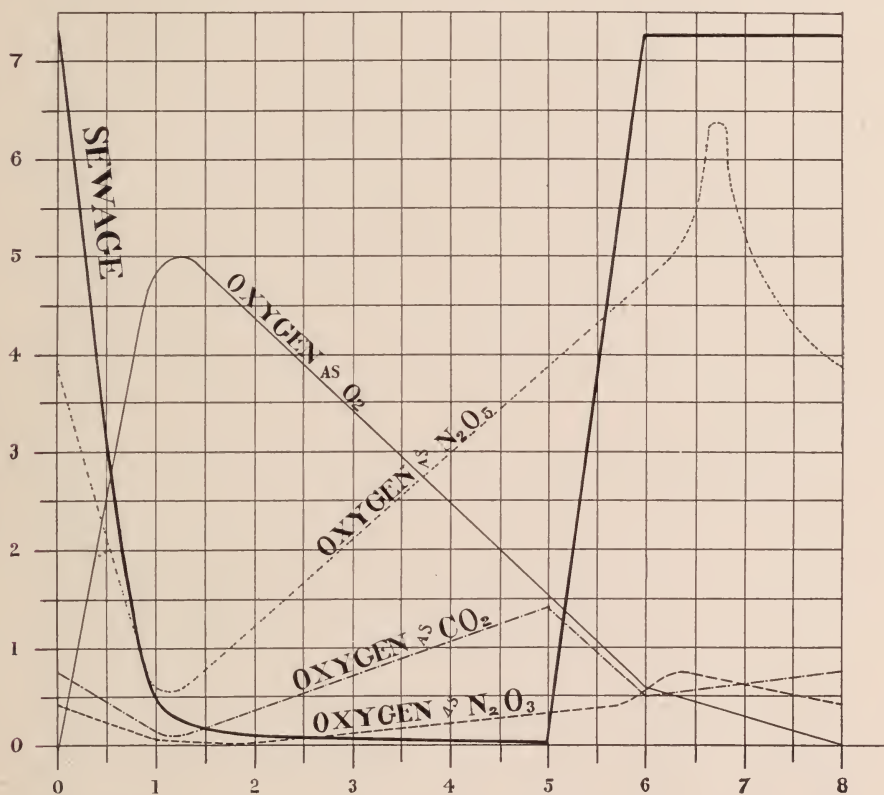
But in spite of all this variation and instability there are certain reactions occurring in a definite sequence which appear to be characteristic of the process as a whole. These salient features, which it is our purpose to point out and discuss, will become most apparent after we have eliminated as far as possible the variable and less essential features by averaging our entire series of results. We may then construct a complete average cycle of the filter, which, while not representing truly the action of the bed at any one time, will nevertheless serve to show the characteristic reactions of each phase, and the relations of these reactions to one another and of the successive phases to the complete cycle.

In order that the data when thus compiled shall be the more



readily understood, we have constructed from it a diagram representing one complete cycle in the operation. The construction of this diagram we will now describe in detail.

On the axis of abscissas are laid off the times of the various stages starting with the emptying phase, one hour, followed by the



oxidation phase of four hours, the filling phase of one hour, and the reduction phase of two hours. As ordinates are laid off the total weights of the oxygen in its various forms, contained in one liter of the open space of the filter. In order to bring the various curves into the same diagram, the ordinate values refer to centigrams of oxygen in the case of the free oxygen and carbon-dioxide, and to milligrams of oxygen in the case of the nitrates and nitrites. The various lines are obtained as follows: The line

representing the total volume of sewage contained in the filter was constructed from Table 7, showing the rate of discharge, and from the known uniform rate of filling. To avoid confusion the ordinate values are not given for this sewage curve. All the other quantities are expressed in terms of oxygen.

The free oxygen curve starts at 0 with the value of the dissolved oxygen in the effluent. This is the average of 45 results given at the five pet-cocks. At 1, the curve would have the value of one-fifth the total capacity of the tank, this value being based on our analysis of the tank-house air; but for convenience in plotting we have subtracted from this the amount of oxygen which is driven from the tank at the time of filling, also determined by analyses, and which therefore takes no part in the reaction. From 1, the line is arbitrarily drawn straight to the point at 6, at which the value is based upon the measurement of the dissolved oxygen in the sewage as it runs in. The value used is the average of 45 determinations made at the five pet-cocks.

The curves for the oxygen as nitrates and nitrites have values at 0, which show the amounts of oxygen in these forms in the effluent. At 1, the values are calculated from the fact that these substances are passing out with the effluent, and on the assumption that the production of nitrates and the loss of nitrites shown in the next period are taking place in this period at a rate proportionate to the amount of the filtering material exposed to the air. At 5, the values are the amounts in the tank immediately on filling. These values are the averages of the 45 determinations at the five pet-cocks. The curves are then carried on over 6 to the maximum point by means of values given in Table VI, and from this point are drawn as a straight line back to 8, which is the same as 0.

The curve showing the oxygen as carbon-dioxide is of quantitative value only between 1 and 5, where it is based upon the gas analyses made during the empty period. The value at 0 is that of a single analysis of the effluent, and at 6, of one analysis of sewage after flowing in.

A study of this diagram, in connection with certain well-known

physical phenomena leads us to the following conception of the action of the contact filter. As the sewage flows into the bed it passes in thin films over the filtering material which in our case was composed of small crushed stones. The stones are covered on their surface with a thin gelatinous growth which, upon microscopical examination, is seen to be a true zoögleal jelly, containing countless numbers of bacteria. In passing over this gelatinous material the sewage is submitted to adsorption which, we believe, is a very important phenomenon in the action of the bed. Adsorption is that property by which certain substances, notably colloids, will remove dissolved material from its solution. It is well known that colloidal precipitates will adsorb certain bases like ammonia from their solutions and the power of charcoal to remove color and odor from water is another example. By this property a large portion of the dissolved substances is removed from the solution and held on the surface of the filtering material. It is this effect upon which Dunbar and Thumm laid so much stress. We believe that we are the first to definitely explain the manner in which this soluble material is withdrawn from the sewage and retained within the filter until it can be acted upon during the oxidation phase.<sup>1</sup>

When the tank is full sedimentation takes place as suggested by Dibdin and the suspended solids are largely removed in this way, but it is obvious that adsorption is by far the more important effect.

When the filter is emptied air is drawn in and brought into contact with the gelatinous film with its contained organic matter. The oxygen also is adsorbed, or dissolved, in the film. This fact we showed in our bottle experiments the results of which are given in Table 3. Not only is it thus brought into intimate contact with the organic matter, but by the well known catalytic effects of colloids, the oxidation of the organic matter by the bacteria is thereby very much hastened. In fact it is just conceivable that a part of this oxidation, at least, is due to the bacteria

<sup>1</sup> For a full discussion of adsorption and especially adsorption of substances in solution by colloids, the following papers may be consulted. VAN BEMMELEN, *Ztschr. f. physik. Chem.* 1895, 18, p. 331; *Ztschr. f. anorg. Chemie*, 1896, 13, p. 233. WALKER AND APPLEYARD, *Jour. Chem. Soc.*, 1896, 69, p. 1334.

only in as much as it is the result of a chemical reaction catalyzed by bacterial colloids. The result of this oxidation is the production in some measure of complete oxidation products, nitrates and carbon dioxide, but more important than these are the partially oxidized products. Certain complex molecules like those of proteid substances if exposed to anaërobic fermentations give rise to malodorous products and the process becomes one of putrefaction. But in the presence of a plentiful supply of oxygen it seems to be the case that such substances are decomposed into simpler molecules and that these latter while not completely oxidized are nevertheless stable and inoffensive. About eight-tenths of the total amount of oxygen used up by the filter can only be accounted for by assuming the formation of such partially oxidized bodies.

These partially oxidized substances are carried off by the next dose of sewage. The nitrates and nitrites are dissolved and give rise at this point to what, in our opinion, is one of the most important reactions of the process. The figures given in Table 5 seem to show that the nitrites remain practically constant during the reduction phase and that the nitrates and the free oxygen disappear. What actually happens, we believe, is this. Nitrification, that is the oxidation of ammonia by the nitrifying organism, is going on all the time, using up the oxygen and forming nitrites and nitrates. Another group of bacteria, the denitrifying bacteria, are bringing about the reverse reaction reducing the nitrates to nitrites. The nitrites are being used up as fast as they are formed in a certain characteristic way which we can best explain by the following reaction:



in which  $R'$  represents certain monovalent organic radicals. In other words, all primary amines, and many amids and amido compounds react with nitrous acid to form free nitrogen, water and an alcohol. The well known reaction between urea and nitrous acid may serve as an example, and, no doubt, in the case of a very fresh sewage that reaction is one of those which take place.



Since this reaction plays an important part in our theory of the action of the filter we will sum up briefly the evidence for its occurrence.

Amines and amids are well known to result from the decomposition of proteid substances, and the reaction is a necessary one if these bodies are mixed with nitrites.

Careful studies of the applied sewages and the effluents of contact filters generally show a considerable loss of nitrogen during the purification. This loss has in most cases been ascribed to a storage of nitrogen within the filter, and no doubt a portion of it is due to that cause. If, however, as has been pointed out by Clark, we base our nitrogen calculations entirely upon that nitrogen existing as free ammonia, nitrates, and nitrites, then any loss observed must be due to an actual loss of nitrogen during the process, since those three forms of nitrogen cannot, as far as we know, be permanently stored. Moreover, since organic nitrogen is being continually converted into ammonia and often oxidized to higher oxides, the loss of nitrogen calculated by this method will always be a minimum value, or, in other words, the actual loss of nitrogen must be greater than is indicated by such a calculation. On the other hand, if we compare the total nitrogen of the effluent with that of the applied sewage, the difference will be a maximum value for the loss of nitrogen; that is, it will be the actual loss of nitrogen plus the nitrogen stored within the filter. We thus have a means of fixing an upper and a lower limit within which must lie the value denoting the actual loss of nitrogen in any purification process.

In four contact filters studied by him, Clark (1903)<sup>1</sup> found for this minimum value a loss of nitrogen of from 38 to 50 per cent. of the total nitrogen applied as free ammonia. From our analyses already given of the raw sewage and effluent of filter 16 a similar calculation may be made. The filter was a new one, and a certain storage of nitrogen must be looked for. Calculating the results of the first six months, using only the free ammonia and the nitrates and nitrites, we find a loss of nitrogen amounting to not less than 29 per cent of the total applied. A series of deter-

<sup>1</sup> *Loc. cit.*

minations recently made of the total nitrogen in the applied sewage and the effluent of this same filter showed a loss of just 50 per cent of the total nitrogen applied, while calculating the loss from the free ammonia and the nitrates and nitrites only, we get a value of 39 per cent. The one is a minimum and the other a maximum value.

These facts are not new, but we believe that they have never been properly explained or sufficiently emphasized. Many of these substituted ammonias, amines, etc., are volatile substances possessing offensive odors. Their complete removal, therefore, at the very beginning of the process is of great advantage, and here, as we see it, lies the chief value of the nitrates and nitrites. In view of the large amount of nitrogen rendered harmless by this reaction, and of the fact that the nitrogenous substances are responsible for the offensive nature of putrefaction, we cannot avoid the conclusion that the reaction between the nitrites on the one hand and the amines and amido bodies on the other is of prime importance in the contact system of sewage purification. It would seem, moreover, that the reaction in question, accompanied by a large loss of nitrogen, is quite characteristic of this system of purification. Clark<sup>1</sup> finds in the case of those other types of filter in which the aëration is more complete, the sand filter and the trickling filter, that the loss of nitrogen is quite small, about one per cent. Results at this station confirm this fact. Averaging the total nitrogen results from our three sand filters, we obtain as a maximum figure for the loss of nitrogen 5.1 per cent. Similarly for three trickling filters the maximum loss of nitrogen is 6.0 per cent. In the case of our three best contact filters, on the other hand, we calculate from the ammonia, nitrate, and nitrite values a minimum loss of nitrogen of 35.6 per cent, while the maximum value, based on the total nitrogen, is 50.2 per cent.

These differences are too important to be overlooked. They indicate two very distinct types of purification: the slow-sand filter and the trickling filter on the one hand, representing a more or less direct oxidation of the organic matter, comparable with direct

<sup>1</sup> *Loc. cit.*

combustion, and the contact filter on the other, a much more complex process, purifying sewage by means of a reaction peculiar to itself, through which more than one-fourth of the total nitrogen of the sewage is set free in the elementary form.

The slow-sand filter gives rise to completely oxidized products, and, as a measure of its efficiency, it is the practice to compare the amount of nitrates in the effluent with the total nitrogen content of the raw sewage. In the contact filter, on the other hand, complete oxidation is not the result attained. We agree with Dunbar that the appearance of nitrates in the effluent is incidental rather than essential to the process. We believe that they are formed regularly in the operation of the filter and that their formation during the oxidation phase is of much more importance than Dunbar supposes, but we cannot accept the view that their appearance in the effluent is in any way a measure of the efficiency of the purification. In fact, in the light of our experimental results, we think that it may very properly be held, that in any two cases the production of nitrates being equal, the ultimate purification will be inversely as the amount of nitrate in the effluent, or in other words, the stability or non-putrefactive character of the effluent depends in considerable degree upon the amount of nitrate *used up*.

Our conception of the action of the contact filter, therefore, may be summed up as follows:

Nitrification takes place within the bed during the greater part of the cycle, but is of special importance during the oxidation phase. This nitrifying action is essential to the successful operation of the bed.

During the reduction phase nitrification continues at a diminishing rate while denitrification is taking place at an ever increasing rate as the conditions become more and more anaërobic. The interaction between the nitrites and the amines and amido bodies taking place at this time, and the accompanying loss of free nitrogen, is one of the most important reactions in the process and is peculiar to the contact system.

The physical phenomenon of adsorption plays an essential part in the action of the bed, serving to remove soluble matter

from the sewage and to place the atmospheric oxygen in intimate—possibly atomic—contact with the organic matter. The adsorbing material—the bacterial zoöglea—thus acts as a contact catalyzing agent in bringing about an intimate mixture of the reacting substances.

Finally, the quality of the effluent is not dependent upon the amount of nitrates contained within it, although an effluent containing nitrates is certainly a good one. In filters of this type it is the nitrate used up that produces the beneficial result. A perfectly stable effluent may contain no nitrates.



## THE DETERMINATION OF THE ORGANIC NITROGEN IN SEWAGE BY THE KJELDAHL PROCESS.

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AT the present time there is evident among sewage analysts a tendency to adopt, as a routine procedure in the analysis of sewage and the effluents of sewage filters, the determination of the total organic nitrogen by the Kjeldahl process, either in conjunction with the albuminoid ammonia process of Wanklyn, or as a substitute for that process. The writer in a paper read before the Laboratory Section of the American Public Health Association at the Washington meeting, in 1903, strongly recommended this change and endeavored to show the untrustworthy nature of the so-called albuminoid ammonia figures. The failure of conversion factors to change these figures into even approximate figures for the total organic nitrogen was especially noted.<sup>1</sup>

The Kjeldahl process then recommended was one which had been used by Palmer<sup>2</sup> in his work on the streams of Illinois, and had up to that time given in our work uniformly good results when applied to sewage. When, however, we came to apply the process to our sewage filter effluents we began to get certain suspiciously low results for which we were unable to account. At times the total organic nitrogen was even lower than the albuminoid ammonia figures. These poor results were always on the better grade of effluents. They did not appear regularly, and when they did occur the duplicate analyses never agreed. At about the same time similar difficulties were reported from other laboratories. In one case the raw sewage did not yield reliable results by the simplified Kjeldahl process, and carefully repeated work showed that the poor figures were not due to faulty manipulation but were inherent in the process.

<sup>1</sup> PHELPS, *Jour. Infect. Dis.*, 1903, 1, p. 327.

<sup>2</sup> *Report on Streams Examinations, Sanitary Dist. of Chicago*, 1902, p. 60.

An experimental study of the Kjeldahl process and its modifications was therefore undertaken in an endeavor to explain the occasional failure of the simplified process which had up to that time been used.

The present paper is a detailed account of these experiments with an analysis of the data obtained. As a result of this work and at the suggestion of the committee of the American Public Health Association on Standard Methods of Water Analysis, a standard method of procedure is outlined which, it is believed, will yield accurate results and which, at the same time, is sufficiently rapid and simple in manipulation to permit its adoption as a routine procedure in sewage analysis.

#### THE KJELDAHL PROCESS AND ITS MODIFICATIONS.

In general there may be said to be two processes for the determination of the nitrogen in an organic compound. First, the combustion method of Dumas, in which the substance is ignited in the presence of cupric oxide and the nitrogen evolved and measured as such; and, second, the Kjeldahl method or one of its many modifications, in which, by treating with boiling concentrated sulphuric acid, the organic carbon is slowly oxidized and the nitrogen obtained in the form of ammonia. The former process is the absolute one. The latter, owing to its simpler nature, is the one upon which we chiefly rely for the determination of the nitrogen in such mixed materials as fertilizers and food-stuffs, and is the only one which can be used for such a liquid mixture as sewage.

A large number of modifications of the Kjeldahl process have been devised, most of which are intended either to shorten the time necessary for the digestion of the organic matter, or to insure the completion of the reaction. To these ends various reagents are used in addition to the sulphuric acid. Such reagents may be conveniently divided into five groups, according to the work they perform in the determination. First, reagents added to raise the boiling point of the acid; second, reagents which will act as catalytic agents, or carriers of oxygen; third, reducing reagents; fourth, reagents added at the conclusion of the digestion to insure the complete oxidation of certain refractory substances; and fifth,

reagents added in the presence of nitrates either to reduce the nitric nitrogen to ammonia and thus include it in the determination, or else to remove this nitric nitrogen in the form of one of the lower oxides.

As an example of the first group of reagents we have potassium sulphate, as used in the Gunning modification of the Kjeldahl process. This salt raises the boiling point of the mixture and materially lessens the time necessary for complete digestion. Incidentally many reagents added for other purposes are present in sufficient concentration to attain this end.

A number of catalytic agents are employed in the various modifications of the process. Mercury, either as the oxide or in the metallic form, is specified in the official method adopted by the Association of Official Agricultural Chemists (Wiley, 1899).<sup>1</sup> Copper, as the oxide or sulphate, has frequently been used (Jodbauer, 1887);<sup>2</sup> (Farnsteiner, Buttenberg, and Korn, 1902);<sup>3</sup> (Brown, 1903).<sup>4</sup> Platinum chloride with copper oxide has been recommended by Ulsch, Proskauer and Zülzer (1889).<sup>5</sup>

The addition of reducing reagents has been found necessary in the presence of any considerable quantity of nitrates, and they have also been found to be essential in the analysis of such bodies as amido-azo-benzene, hydroxylamine, and many others (Dyer, 1895).<sup>6</sup> Zinc dust, salicylic acid, and sugar have been used for this purpose.

Of reagents added at the end of the digestion to complete the process potassium permanganate is most commonly employed. Ward (1894),<sup>7</sup> showed that there were certain organic compounds, generally those containing a benzene ring, which did not yield to the ordinary treatment, however prolonged, but which were readily

<sup>1</sup> WILEY, U. S. Dept. of Agriculture, Bureau of Chemistry, *Bull. No. 46*, Washington, 1899.

<sup>2</sup> JODBAUER, *Ztsch. f. anal. Chem.*, 1887, 26, p. 92.

<sup>3</sup> FARNSTEINER, BUTTENBERG AND KORN, *Leitfaden für die chemische Untersuchung von Abwässer*. München and Berlin, 1902.

<sup>4</sup> BROWN, J. W., *Kinetische Studien über katalitsche Beschleunigungen bei der Oxydation von Naphthalin und Analinsulphate durch heisse Schwefelsaure*. Heddleburg, 1903.

<sup>5</sup> ULSCH, PROSKAUER AND ZÜLZER, *Ztschr. f. Hyg.*, 1889, 7, p. 216.

<sup>6</sup> DYER, B., *Jour Chem. Soc.*, London, Trans. 1895, 67, p. 811.

<sup>7</sup> WARD, *The Determination of Nitrogen in the Wet Way*. Unpublished thesis. Massachusetts Institute of Technology, Chemical Department, 1894.

and completely oxidized by the addition of a few crystals of permanganate to the hot acid.

Two kinds of special reagent are used in the presence of nitrates. The first has already been mentioned under the reducing reagents. Such substances as phenol and salicylic acid are nitrated by the nitric acid, and may be subsequently reduced by zinc dust to an amine which will yield ammonia on digestion. Such a treatment will include the nitric nitrogen in the final result. A second method aims to reduce the nitric acid to one of the lower oxides and thus exclude it from the result. For this purpose a mixture of sodium bisulphate and ferric chloride has been used.

#### EXPERIMENTAL STUDIES.

The failure to obtain as ammonia the total amount of organic nitrogen in the sample of sewage may be due to one or both of two causes: first, the escape of volatile nitrogenous bodies during the evaporation and digestion; and, second, incomplete digestion of the organic matter.

a) *The loss of nitrogen.*—The question of the volatilization of nitrogenous matter during the digestion is a somewhat complicated one. Owing to the basic nature of most of the nitrogenous bodies found in proteid decomposition products and to the non-volatile nature of their salts one would hardly expect any direct loss of the organic matter as such. Some experiments have been carried out, however, to show to what extent we are justified in making such an assumption.

To this end regular Kjeldahl determinations were carried out upon samples of raw and septic sewage, and upon the effluents of various sewage filters. In each case the flask was connected with a Liebig condenser, and in this way all the water of the sample, together with part of the sulphuric acid and any volatile matter which distilled over was condensed. This distillate was subsequently treated with strong alkaline permanganate and redistilled, boiling almost to dryness in order to recover, in large part at least, any organic nitrogen which had escaped during the evaporation and digestion of the sample. The results of these determinations are shown in Table 1.



TABLE 1.  
SHOWING THE AMOUNT OF ORGANIC NITROGEN LOST THROUGH VOLATILIZATION DURING THE EVAPORATION AND DIGESTION OF THE SAMPLE.

Sample	PARTS PER MILLION	
	Organic Nitrogen	Nitrogen Recovered in Distillate
Fresh sewage .....	18.5	0.10
Septic sewage .....	17.3	0.13
Effluent, contact filter ..	9.0	0.06
Effluent, sand filter .....	1.7	0.00

These figures seem to indicate that there occur in the sewage and effluents certain organic nitrogenous bodies which are either volatile at the temperature of the boiling liquid or, at least, are capable of being distilled over with the steam. The amount of nitrogen thus escaping may be considered roughly as about one per cent of the total, and this value seems to be fairly constant for the various classes of water studied. It is an entirely negligible quantity, and no method is readily apparent by which the loss can be avoided. In certain abnormal sewages, and especially in waste waters from factories, it is conceivable that a more serious loss may occur in this manner. In any such case it would be advisable carefully to determine this point before proceeding with the nitrogen determination.

There still remains the possibility of the formation of one of the lower oxides of nitrogen, or of free nitrogen itself, during the digestion. In this case there would be a loss of nitrogen which would not be detected in the experiments just quoted, and which, indeed, owing to its very small volume, it would be almost impossible to detect by any direct means. To assume, however, without further evidence that all organic nitrogen, in whatever form it may exist in the molecule, will invariably appear in the form of ammonia after digestion would be quite unwarranted. Fortunately, there is a large amount of experimental evidence bearing out this assumption.

The agricultural chemists throughout the country have made extensive studies of the Kjeldahl process as applied to fertilizers.

In most of this work direct comparisons have been made between this process and the absolute method of Dumas. As a result of these studies, extending over many years, an official Kjeldahl method has been established which is supposed to yield results closely approximating the true nitrogen values. Owing to the somewhat similar natures of the organic matter of fertilizers and that of sewage, this work is of considerable value to the sewage analyst.

In addition to this work upon fertilizers certain exhaustive studies have been made upon the Kjeldahl process as applied to pure substances of known composition. This work all tends to show that under proper treatment the nitrogen of a large number of organic compounds can be recovered quantitatively as ammonia. Dyer (1895),<sup>1</sup> in a study of over 30 compounds, covering a wide range of organic types, obtained satisfactory results in the majority of cases by simply digesting with sulphuric acid in the presence of mercury and potassium sulphate. In certain other cases reducing agents were necessary, and in only one case was he unable to obtain the whole of the nitrogen with a fair degree of accuracy. Sodium nitroprusside gave results about 10 per cent too low. Similar results are reported by Bosshard (1884)<sup>2</sup> and by Arnold and Wedemeyer (1892).<sup>3</sup>

It still remains to be shown that in sewage there are no substances which will complicate the reaction and in some way eliminate a portion of the nitrogen. Such a reaction, for instance, as that which occurs between nitrites and primary amines would give rise to a loss of free nitrogen and introduce a serious error in the determination. Three substances may occur in a normal sewage or in the effluents of sewage filters which might possibly lead to such an indirect loss of nitrogen. These are chlorides, nitrites, and nitrates.

There can be hardly any question that these substances might react with the nitrogenous matter of the sewage in such a way as to cause loss of nitrogen. Nitrites may react with amines as indicated above. The possible effect of nitrates is not so readily stated. The present evidence seems to show that the well known

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Ztschr. f. anal. Chemie*, 1884, 24, p. 199.

<sup>3</sup> *Ibid.*, 1892, 31, p. 525.

rapid destruction of nitrates by sewage is due to bacterial action. (Gayon and Dupetit, 1886;<sup>1</sup> Ampolo and Ulpiani, 1898;<sup>2</sup> Letts, 1904.<sup>3</sup>) Chlorides are always present at the same time, and the interaction of the nitric and hydrochloric acids produced will give rise to a certain amount of free chlorine. Experiments made by the writer have shown that, in common with many other oxidizing reagents, free chlorine will cause a loss of nitrogen in the Kjeldahl process. The loss is doubtless due to a reaction similar to that between urea, chlorine and water, viz.:



The question therefore is whether these substances in the quantities in which they are likely to be met with in sewage and sewage effluents are sufficiently concentrated to produce a noticeable loss of nitrogen during the determination. To answer this question the following series of experiments was made.

A series of determinations was first made to study the effect of chlorides. In each case a nitrogen determination was made on the original sample. Enough sodium chlorid was then added to double the original chlorine content and a second determination was made. The procedure followed was the one to be described in another part of this paper as the standard method. The results of the series are shown in Table 2.

TABLE 2.

SHOWING THE EFFECT UPON THE DETERMINATION OF THE TOTAL ORGANIC NITROGEN OF THE ADDITION OF CHLORIDES TO THE SAMPLE.

SAMPLE	Chlorine	NITROGEN AS			
		Free Ammonia	Albuminoid Ammonia	Total Organic by Kjeldahl, Original Sample	Total Organic by Kjeldahl, After Doubling the Chlorine
Fresh sewage.....	3,600	15.0	3.5	10.0	10.0
Septic sewage.....	1,520	17.5	3.3	8.5	8.5
Effluent, contact filter	4,000	15.0	3.7	7.5	7.5
Effluent, sand filter..	2,700	4.0	0.6	6.0	6.0

<sup>1</sup> *Station Agronomic de Bordeaux*, 1886.

<sup>2</sup> *Gaz. chim. ital.*, 1898, 28, p. 410.

<sup>3</sup> *Sanit. Rec.*, London, 1904, n. s. 33, pp. 488, 536, 552; 34, p. 136.

The excessive chlorine values are caused by the leakage of sea-water into the sewer from which the sewage was pumped. To guard against the possibility that the large amount of chlorid already present in the sample was sufficient to cause the greatest possible loss of nitrogen during the digestion, and that therefore the addition of more chlorid would not increase this loss, the two effluents noted in Table 2 were treated in the following manner. The chlorine of each sample was precipitated by the addition of an excess of solid silver sulphate to the hot sample which had been previously acidified with sulphuric acid. After boiling for a minute, the precipitated silver chlorid was allowed to settle. The nearly clear supernatant liquor was poured off and the silver chlorid was dissolved by prolonged boiling in sulphuric acid. When solution was complete this acid was added to the original solution and the digestion was carried out as usual. The two effluents mentioned gave under this treatment results identical with those given in the table.

It appears therefore that chlorides, *per se*, even when present in such excessive quantities as are found in the sewage used in these experiments, exert no harmful influence upon the determination.

In a similar way the effect of the addition of nitrates and nitrites to the sample was studied. Solutions of these substances were added to the samples in varying amounts and the total organic nitrogen was then determined. These solutions were of such a strength that one cubic centimeter contained one-tenth of a milligram of nitrogen, so that each cubic centimeter added to the sample of 100 c.c. represented one part of nitrogen per million. The composition of these samples and the amount of organic nitrogen found in each case are shown in Table 3.

These results show conclusively that the presence of even considerable amounts of nitrites, nitrates and chlorides does not give rise to any serious loss of nitrogen during the treatment. Taken in conjunction with the results of other workers cited here they seem to indicate that in so far as the nitrogen is released from the organic molecule it can be recovered quantitatively as ammonia. They also show about what accuracy may be looked for



in this process. The process as carried out does not permit of readings any closer than five-tenths of a part per million, on a sewage or an effluent of poor quality. Such an accuracy is probably all that is warranted, owing to the difficulty of obtaining in

TABLE 3.  
SHOWING THE EFFECT UPON THE DETERMINATION OF THE TOTAL  
ORGANIC NITROGEN OF THE ADDITION OF NITRATES  
AND NITRITES TO THE SAMPLE.

A.—*Sample of Fresh Sewage.*

Analysis. Parts per million.

Nitrogen as free ammonia, 18.5; albuminoid ammonia, 3.5; nitrites, 1.5; nitrates, 0.0; chlorine, 8.10.

PARTS PER MILLION		
Nitrogen Added as Nitrate	Nitrogen Added as Nitrite	Organic Nitrogen Found
0	0	5.5
1	0	6.0
5	0	5.0
10	0	5.0
0	1	5.5
0	5	5.5
0	10	5.5
5	10	5.5
10	5	5.5

B.—*Sample of Septic Sewage.*

Analysis. Parts per million.

Nitrogen as free ammonia, 22.5; albuminoid ammonia, 3.0; nitrites, 0.0; nitrates, 0.0; chlorine, 1.570.

PARTS PER MILLION		
Nitrogen Added as Nitrate	Nitrogen Added as Nitrite	Organic Nitrogen Found
0	0	8.0
1	0	8.0
5	0	8.0
10	0	8.0
0	1	9.0
0	5	8.5
0	10	9.0
5	10	9.0
10	5	8.5

TABLE 3—Continued.

## C.—Sample of a Contact Filter Effluent.

Analysis. Parts per million.

Nitrogen as free ammonia, 15.0; albuminoid ammonia, 4.4; nitrites, 0.16; nitrates, 5.3; chlorine, 950.

PARTS PER MILLION		
Nitrogen Added as Nitrate	Nitrogen Added as Nitrite	Organic Nitrogen Found
0	0	7.0
3.5	0	7.0
0	0.16	7.0
3.5	0.16	7.1

## D.—Sample of a Sand Filter Effluent.

Analysis. Parts per million.

Nitrogen as free ammonia, 0.53; albuminoid ammonia, 0.12; nitrites, 0.2; nitrates, 40.0; chlorine, 1,400.

PARTS PER MILLION		
Nitrogen Added as Nitrate	Nitrogen Added as Nitrite	Organic Nitrogen Found
0	0	1.52
40.0	20.0	1.48

a small volume a proper sample of such a heterogeneous mixture. It is undoubtedly this difficulty that gives rise to the irregularity of the results rather than anything inherent in the process. These irregularities are so scattered, however, that it is apparent that the substances added have exerted no influence upon the results.

b) *The digestion.*—We have then to consider the second point mentioned as a possible source of error in the process, namely, incomplete digestion. It is readily apparent that if the digestion is not complete there may be and probably will be some nitrogen left in the organic form at the end of the process which would not be recovered as ammonia. The official method of the agricultural chemists already referred to calls for a digestion until "the contents of the flask have become a clear liquid which is colorless or at at least only a very pale straw color." Other authorities

allow a "pale yellow color." The writer is convinced that in the various interpretations of the proper end-point of the digestion lies much of the difficulty of obtaining correct results. Especially is this the case when potassium permanganate is used at the conclusion of the digestion. This point was shown in a striking way by some experiments made in an attempt to hasten the process of digestion by the addition of oxydizing agents. It was found that the addition of either potassium permanganate, potassium bichromate, or free chlorine previous to the digestion gave rise to a very considerable loss of nitrogen. The following experiments are selected to illustrate this effect in the case of permanganate:

*Experiment 1.*—Sample, raw sewage.

Parts per million of nitrogen as	{	Free ammonia . . . . .	20.0
		Albuminoid ammonia . . . .	4.0
		Nitrites . . . . .	0.0
		Nitrates . . . . .	0.0
		Total organic . . . . .	13.5

One hundred c.c. of this sewage was treated with 5 c.c. of potassium permanganate solution (.4 gm. per litre), and a few drops of sulphuric acid and boiled for 10 minutes. It was then treated with sulphuric acid and digested as usual. The organic nitrogen recovered was 8.5 parts per million.

*Experiment 2.*—Sample, sand filter effluent.

Parts per million of nitrogen as	{	Free ammonia . . . . .	2.4
		Albuminoid ammonia . . . .	1.4
		Nitrites . . . . .	14.0
		Nitrates . . . . .	20.0
		Total organic . . . . .	7.6

This sample was treated as in Experiment 1. Total organic nitrogen found, 2.6 parts per million. A second portion was warmed for 30 minutes with the permanganate solution. Total organic nitrogen found, 5.6 parts per million.

Similar results were obtained by the addition of potassium bichromate and of aqua regia. The results show clearly that the addition of such oxidizing reagents before the digestion is complete will be accompanied by a decided loss of nitrogen. The

reason for this loss is not clear. In the case of the slow oxidation of the organic molecule by sulphuric acid the carbon is evidently first attacked and the nitrogen is left in the form of ammonia which is incapable of further oxidation either by the acid or by permanganate. In the more violent oxidation of the organic molecule by the permanganate it may be possible that the hydrogen which is attached to the nitrogen is first oxidized, leaving N-O groups which are subsequently liberated and escape as one of the lower oxides of nitrogen.

In order to study the effect of the permanganate upon a sample which is almost completely digested and also to show the comparative results obtained by digesting samples to the different end-points which have been mentioned, the following series of analyses was made. Four samples were used. Upon each sample three determinations of the total organic nitrogen plus the free ammonia were made. In the first case the digestion was carried to a point at which the clear solution had a "pale yellow color," as interpreted by three members of the laboratory staff; in the second case the color obtained was "very pale straw," and in the third case the digestion was carried out until the color was entirely gone. Permanganate was added at the conclusion of each digestion as in the standard procedure. The following results were obtained:

TABLE 4.  
SHOWING THE EFFECT OF INCOMPLETE DIGESTION UPON THE AMOUNT  
OF NITROGEN RECOVERED.

SAMPLE.	PARTS PER MILLION OF ORGANIC PLUS AMMONIACAL NITROGEN		
	Pale Yellow	Very Pale Straw	Colorless
Fresh Sewage.....	28.5	30.0	30.0
Septic Sewage.....	25.0	26.0	31.0
Contact filter, 16.....	9.5	10.0	11.0
Contact filter, 13.....	10.0	11.0	14.0

It is evident from these figures that a colorless solution must be obtained before the digestion may be said to be complete, and that even the "very pale straw color" is not a satisfactory end-point in the case of most samples.



While it has been shown that the addition of the permanganate at an early stage of the determination will give rise to a loss of nitrogen, yet it is true that the use of this reagent at the conclusion of the digestion, will, in some cases at least, release a certain amount of organic nitrogen which has withstood the action of the acid, giving rise to an increased nitrogen value. Ward (1884),<sup>1</sup> states that compounds containing a benzene ring cannot be completely oxidized by the action of the acid alone, but that the addition of permanganate will always complete the oxidation. In Table 5 is given a list of duplicate analyses by the standard procedure except that on one sample in each case permanganate was not used. It will be seen that in many cases there is no increase in the nitrogen value due to the addition of permanganate, while in others the increase is considerable. The use of permanganate is therefore advisable although it is not essential in all cases.

TABLE 5.

SHOWING THE EFFECT OF THE ADDITION OF POTASSIUM PERMANGANATE AT THE CONCLUSION OF THE DIGESTION UPON THE AMOUNT OF ORGANIC NITROGEN RECOVERED.

Parts per Million

SAMPLE	NITROGEN AS			
	Free Ammonia	Albuminoid Ammonia	Organic Nitrogen	
			With Permanganate	Without Permanganate
Sewage.....	15.0	3.5	10.0	8.0
Sewage.....	20.0	7.1	24.0	19.0
Sewage.....	17.5	6.3	23.0	21.0
Septic sewage.....	22.5	4.2	12.5	12.5
Contact filter Effluent....	12.4	2.1	4.7	4.2
Sand filter Effluent.....	1.6	1.0	2.5	2.5
Septic tank Sludge.....	65.0	22.0	122.0	122.0

The time necessary to accomplish digestion may often be longer than is consistent with the routine analysis of a large number of samples. The question of the use of a catalytic agent has therefore received considerable attention in this study. Of those metallic salts which have been recommended for this

<sup>1</sup> *Loc. cit.*

purpose by various workers copper sulphate was selected as being the one most satisfactory from many points of view. Brown (1903),<sup>1</sup> has shown in a careful quantitative study of the catalytic acceleration of the oxidation of aniline and naphthaline by sulphuric acid that copper and mercury rank first among such catalytic agents and that they are about equal in efficiency. Copper sulphate besides being the cheaper and more convenient substance to use, possesses the additional advantage over mercury that it does not require the addition of sodium sulphid to the solution before distillation. The contrary opinion is often expressed but the writer is satisfied after a careful study of this point that if sodium carbonate is used for neutralization the ammonia can be completely recovered from the solution without the addition of the sulphid.

c) *Minor details.*—As to the amounts of the sample and of the reagents to be employed the attempt has been to employ as large a volume as convenient of the sewage and as small an amount as possible of the other reagents. This practice in the first place allows of a more accurate sampling of the sewage or effluent than if smaller amounts are taken and at the same time reduces the effect of the “blank” due to the reagents to a minimum. I have therefore used, in general, 100 c.c. of the sample and 5 c.c. of sulphuric acid. With these quantities and ordinary pure reagents the blank for the reagents may be entirely neglected.

Having in view the advantages of a process made as simple as possible by the use of few reagents, it has not seemed advisable to attempt to recover the nitric nitrogen by the Kjeldahl process. This may be done if desired by the official agricultural method already cited. In water and sewage work the nitrate determination can be made in a much simpler manner, and indeed would always be so made even if it were also included in the total nitrogen determination. Nitrates will never be found in sewage or sewage effluents in such quantities as to interfere with the process by their own reduction, and this is the chief reason for their determination with the organic nitrogen in fertilizer analysis.

<sup>1</sup> *Loc. cit.*

## PROPOSED STANDARD METHOD.

The following is recommended as a standard method of procedure for the determination of the total organic nitrogen in sewage and in the effluents of sewage purification processes.

## APPARATUS REQUIRED.

*Kjeldahl digestion flasks.* These should be of Jena glass and of about 200 c.c. Flasks having an egg-shaped or oval bulb are preferable to those with spherical bulbs, because they will permit of carrying out the digestion in smaller volume without danger of cracking.

*Stills.* The ordinary indirect stills such as are used in the analysis of sewage, are to be preferred although the direct still may be used when more convenient.

## SOLUTIONS REQUIRED.

*Sulphuric Acid.* Chemically pure sulphuric acid of about 1.83 s.g.

*Sodium Carbonate.* A saturated solution of sodium carbonate. Live steam is blown through this solution until it gives no test for ammonia with the Nessler solution.

*Nessler solution* and a *standard solution of ammonium chloride* such as are used in the determination of ammonia.

*Copper sulphate* and *potassium permanganate.* Chemically pure crystals.

## THE DETERMINATION.

One hundred c.c. of the sample are placed in a digestion flask. Five c.c. of sulphuric acid and a small crystal, about 0.1 gm., of copper sulphate are added and the flask is placed over the flame and boiled down briskly until the contents begin to darken. The flame is then turned down until only a gentle simmering occurs. The digestion is allowed to proceed until the yellow color of the contents of the flask has entirely disappeared. With practice this point can be determined in spite of the green color of the copper salt. If any doubt exists the flame may be removed for a minute when the copper salt will at once settle out perfectly colorless. When the digestion is complete the flame is removed and small crystals of permanganate are added one at a time until the green

precipitate is permanent. The flask is then allowed to cool. When cool the contents of the flask are made up to 500 c.c. with water. Ten c.c. (or more, according to the amount of the nitrogen in the sample), are removed, placed in the distilling flask, diluted with an equal volume of ammonia free water, and neutralized by the addition of 10 c.c. (or an amount equal to the amount of the acid mixture taken), of the sodium carbonate solution. This is a large excess and no indicator need be used. Fifty c.c. are then distilled over by blowing steam through the flask, and the

TABLE 6.

ANALYSES OF BOSTON SEWAGE AND OF THE EFFLUENTS OF SEWAGE FILTERS MADE AT THE SANITARY RESEARCH LABORATORY AND SEWAGE EXPERIMENT STATION OF THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY.

SAMPLE	PARTS PER MILLION OF NITROGEN AS					
	Ammonia		Nitrates	Nitrites	Organic Nitrogen	
	Free	Albuminoid			I	II
Fresh sewage...	20.0	6.1	0.5	0.20	13.5	13.0
	20.0	5.3	0.0	0.18	14.0	14.0
	20.0	6.6	0.2	0.0	14.0	14.0
	22.5	7.0	0.0	0.20	13.5	14.0
	23.5	6.5	0.0	0.00	13.0	13.0
	19.5	5.0	0.0	0.20	12.0	11.5
	16.0	5.0	0.0	0.20	11.0	11.0
Septic sewage...				0.16		
	19.0	2.1	0.0	0.0	4.5	4.8
	23.5	2.7	0.0	0.0	5.5	5.5
Contact filter effluents.....	25.0	2.7	0.0	0.0	9.0	8.0
	20.0	3.0	0.0	0.20	3.5	4.0
	23.5	2.1	0.0	0.0	2.3	2.5
	10.0	1.4	0.8	0.10	4.0	4.0
	19.0	4.8	0.0	0.01	11.0	10.0
	25.0	2.0	0.0	0.0	7.5	7.0
	25.0	2.6	0.0	0.20	6.5	7.0
Trickling filter effluents.....	12.5	3.6	0.0	0.10	15.5	14.5
	15.0	4.2	0.0	0.16	7.0	7.0
	22.5	3.7	5.0	0.60	9.0	8.5
	17.5	4.5	0.0	0.04	6.5	5.5
	14.5	3.7	0.2	0.20	8.0	7.5
Sand filter effluents.....	17.5	4.2	2.0	0.16	9.5	9.5
	19.0	3.7	6.0	3.0	7.0	7.5
	0.52	0.12	40.	0.20	1.48	1.55
	0.80	0.24	30.	0.08	1.8	1.6
	1.2	0.30	35.	0.16	2.8	3.0



ammonia is determined in the distillate in the usual manner by nesslerization.

## CALCULATION.

With samples containing from 10 to 40 parts per million of ammonial and organic nitrogen 10 c.c. of the diluted acid mixture is the proper amount for distillation. The distillate may be directly nesslerized. If the ammonia standard used has the usual strength (1 c.c. = 0.00001 gm. nitrogen), then each cubic centimeter of the standard used represents five parts per million of nitrogen in the sample. Multiplying the reading by five and subtracting from the product the free ammonia value, the result is the total organic nitrogen, expressed in parts per million.

The analyses of sewage and of the effluents of sewage filters of various types which have been made at the Sanitary Research Laboratory and Sewage Experiment Station of the Massachusetts Institute of Technology are given in Table 6. The sewage used is drawn from the trunk sewer of the Boston Main Drainage system.

TABLE 7.

SAMPLE	PARTS PER MILLION OF NITROGEN AS			
	Free Ammonia	Albuminoid Ammonia	Total Organic	
			I	II
Boston—				
Albany St. sewer*.....	3.0	5.3	18.5	18.0
Dartmouth St. sewer*.....	4.0	7.3	22.0	22.0
Brockton, Mass.—				
Stale sewage.....	45.0	8.0	17.5	17.5
Sewage sludge.....	75.	225.	415.	420.
Effluent, sand filter.....	0.24	0.12	0.62	0.60
Concord, Mass.* .....	9.0	5.0	16.0	15.5
Lawrence, Mass.—				
Sand filter No. 1.....	0.068	0.084	0.40	0.43
" " " 5.....	.....	.....	0.73	0.69
Pride's, Mass.—				
Private septic tank. Effluent ....	21.0	6.6	11.5	.....
Worcester, Mass.....	37.5	7.7	22.5	22.5

\* Small sewers. Very fresh sewage.

The analyses are given to indicate the probable accuracy of the method proposed and to show the rough relation existing between the total organic nitrogen and the albuminoid ammonia.

To test more fully the proposed method certain samples were collected from outside sources and submitted to analysis. The writer is indebted to Professor L. P. Kinnicutt, Mr. H. W. Clark, and Mr. G. E. Bolling for assistance in the collection of samples from Worcester, Lawrence, and Brockton respectively. A description of these samples and the analytical results obtained are given in Table 7.

## TESTS OF A METHOD FOR THE DIRECT MICROSCOPIC ENUMERATION OF BACTERIA.

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A METHOD for the direct microscopic enumeration of bacteria in sewage was devised by one of us (C.-E. A. W.), during the summer of 1903. The development of the method and the results of its application to sewage effluents are fully described elsewhere (see p. 41). In general, however, it may be said that the microscopic count indicated the presence of from 10 to 100 times as many bacteria as develop upon the gelatin plate, or even upon the Nährstoff agar medium. This result was so striking that it seemed to us worth while to study, in a little more detail, the relation between the counts obtained by the visual and the cultural methods, with a view to determining the real significance of the higher numbers apparent under the microscope and the practical value of the new process of enumeration.

The microscopic method as finally developed is as follows: After the usual shaking, a sample is withdrawn from the bottle in a sterile graduated one c.c. pipette and one-twentieth of a c.c. is allowed to flow upon a clean square cover-slip. Cover-slips should be boiled in a 10 per cent solution of potassium bichromate in 50 per cent sulphuric acid, and allowed to lie in this cleaning mixture. Just before using they may be rinsed in 50 per cent alcohol and dried on a silk cloth, not in the flame. One-twentieth of a c.c. of water placed on such a cover-slip spreads evenly and should be allowed to dry in the air without too sudden heating. After drying, it is fixed by passing through the flame; covered with Ziehl-Nielsen's carbolfuchsin, warmed till steam just rises, washed, dried, and mounted.

For counting the bacteria we used a Sedgwick-Rafter eyepiece micrometer, made for the study of the larger micro-organisms in drinking water. It bears a large square divided

With practice the microscopic count may be made with considerable rapidity. The sampling of 10 sewages and effluents and the spreading of the samples on the cover-slips take about



20 minutes; the staining and mounting after two hours' drying in the air take 15 minutes, and the counting 30 minutes. The actual time devoted to the analysis of each of the 10 samples is thus less than seven minutes. The fact that results can be reached in one or two hours, instead of 24, might also prove of importance under certain conditions.

TABLE 2.  
VARIATIONS IN NUMBER OF BACTERIA IN SAMPLES OF SEWAGE AND EFFLUENT  
ANALYZED IN DUPLICATE.

Sample	Number of Bacteria Counted in 10 Squares	Average	Average Devia- tion	Average Percentage of Error
Trickling Filter	180 148 188 168 148 168	168	12	7.
Septic Tank...	280 292 240 232 262 152 244 172 280	236	36	15.
Sewage .....	544 876 528 652 628 716 624 680 468	636	84	13.

Obviously the microscopic method of counting is applicable only to substances containing considerable numbers of bacteria. When the number of fields to be examined is very large the process becomes both tedious and inaccurate, and it will probably not be useful for the counting of bacteria in numbers less than 25,000 per c.c.

The high numbers of bacteria found by this method as compared with the ordinary plate count might be due to any or all of three causes. First, even the most careful shaking fails wholly to separate chains and groups of organisms into their constituent cells; hence colonies on a plate sometimes represent not individuals but pairs or larger aggregations of bacteria. Second, the presence of the dead bodies of bacteria in a stainable condition would make the microscopic count larger than the plate count. Third, there may be present organisms which do not grow upon our ordinary nutrient media, or representatives of groups ordinarily appearing which, though still living, are too weak to thrive under the peculiar conditions of a solid culture. With respect to the first and third factors the microscopic count is superior to the plate count, while with regard to the second it fails to give a correct idea of the total number of living bacteria

present. Upon the relative importance of these three factors then the value of the microscopic method must depend.

The extent to which unseparated groups of bacteria introduce an error in the ordinary plate count depends, of course, largely upon the thoroughness with which shaking is carried out. In our first experiments with pure cultures we found very marked differences between the microscopic count and the plate count, due mainly to this cause. This was particularly the case when a suspension was made from the surface growth upon some solid medium, since in this case the bacteria occur in close aggregates very hard to break up. Table 3 illustrates this point.

TABLE 3.

COMPARISON OF MICROSCOPIC COUNT AND PLATE COUNT. AQUEOUS SUSPENSIONS OF *B. SUBTILIS* FROM AGAR STREAK KEPT AT 20°.

Number of hours from beginning of experiment...	0	3	7	24	32
Bacteria per c.c. microscopic count .....	2,300,000	1,000,000	1,500,000	9,000,000	5,600,000
Bacteria per c.c. gelatin plate .....	450,000	260,000	540,000	3,000,000	1,000,000

TABLE 4.

COMPARISON OF MICROSCOPIC COUNT AND PLATE COUNT. *B. SUBTILIS* IN DILUTED BROTH.

Number of hours from beginning of experiment .....		0	6	24	48	72
A. at 20°	Bacteria per c.c. microscopic count...	4,500,000	9,300,000	21,000,000	14,500,000	11,900,000
	Groups of bacteria, per c.c. microscopic count. ....	3,000,000	8,000,200	15,000,000	6,600,000	9,000,000
	Bacteria per c.c. gelatin plate.....	3,000,000	7,000,000	500,000	1,000,000	6,500,000
B. at 15°	Bacteria per c.c. microscopic count....	5,000,000	10,200,000	6,300,000		
	Groups of bacteria per c.c. microscopic count.....	3,600,000	6,200,000	4,500,000		
	Bacteria per c.c. gelatin plate.....	7,000,000	770,000	2,500,000		

Table 4 shows the comparative counts obtained by diluting a broth culture with water. Here the results obtained are much better, with the exception of three instances in which the gelatin

count was abnormally low. Separate counts are recorded for the individual cells and for the groups of bacilli under the microscope, and they show that the number of cells was from 10 to 100 per cent higher than the number of groups. In Table 10 the same thing is shown in more detail. In this experiment suspensions were made in diluted broth of streak cultures of *Micrococcus roseus* and inoculated with a small portion of surface growth from a streak of *B. subtilis*. From the table it appears that in spite of very thorough shaking both cocci and bacilli adhered so closely together that the number of cells was often double the number of groups. In the examination of sewage and sewage effluents by the microscopic method it is common to find pairs and even long chains of cocci and bacilli, and irregular groups of organisms held together by a sort of zoöglöea. In this respect the microscopic count gives a truer idea of the number of bacteria present than does the plate method.

The presence in the fluid to be analyzed of large numbers of dead bacterial cells in such a condition as to be fixed and stained by carbolfuchsin would, on the other hand, introduce a serious error in the microscopic count; and this possibility appears to us the most serious theoretical objection to the method. We know, indeed, from the study of higher organisms that protoplasts after death degenerate and break up so that definite cell structures can no longer be made out by treatment with anilin dyes. Whether under the conditions which obtain in organic fluids this process is sufficiently rapid to prevent the accumulation of a large number of stainable dead cells is the question upon which the value of the microscopic count depends.

The fact that dead bacteria do lose their staining property is made clear by the examination of any old solid culture. We have, for example, made a suspension from an old culture of *B. typhosus*, which, when stained and mounted, showed the field crowded with rods so faintly tinged as to be barely visible. On the other hand, under certain conditions, dead cells may retain their normal condition for a considerable period. In a sterilized pure culture, for example, the bacteria are still stainable after many hours, as shown in Table 5.

TABLE 5.

MICROSCOPIC COUNTS OF AQUEOUS SUSPENSION OF *B. SUBTILIS* BEFORE AND AFTER HEATING TO 70°-80° FOR 30 MINUTES.

Time	Before Heating	After Heating	2 Hours After Heating	4 Hours After Heating	6 Hours After Heating	24 Hours After Heating
Bacteria per c.c. (microscopic count).....	23,900,000	19,200,000	22,500,000	26,900,000	20,100,000	20,900,000
Bacteria per c.c. agar, 37°.....	3,360,000	19	0	0	0	0

Sterilization, by heat and by most chemical agents, however, probably involves a coagulation of the protoplasm, thus in a way fixing and protecting it from the normal changes of decomposition. In this respect the test is not a fair one; and we next used a suspension in which the bacteria were killed by exposure to direct sunlight. Again the dead cells persisted for many hours as shown in Table 6.

TABLE 6.

MICROSCOPIC COUNT OF AQUEOUS SUSPENSION OF *B. SUBTILIS* BEFORE AND AFTER EXPOSURE TO DIRECT SUNLIGHT.

Time	Before Exposure	After 2 Hours' Exposure	After 5 Hours' Exposure	20 Hours After Removal from Sunlight
Bacteria per c.c. (microscopic count) .....	22,000,000	27,200,000	29,600,000	15,400,000
Bacteria per c.c. (agar, 37°) ..	950,000	147	60	770,000

Still our conditions did not duplicate those which actually obtain, since in sewage and other decomposing fluids dead bacteria occur in the presence of other living and actively multiplying cells; and the products of growth of the latter might be expected to exercise an important effect upon the dissolution of the former. We first tested this point by sterilizing septic sewage in the autoclave and mixing with it one-tenth its volume of untreated septic sewage. Thus a large number of dead cells were brought into contact with a smaller number of living bacteria under such conditions that the latter could multiply freely. The results shown in Table 7 indicate a destruction of three-fifths of the total



of living and dead bacteria present during the first three hours after the samples were mingled signifying the dissolution of 30,000,000 cells per c.c.

TABLE 7.

MICROSCOPIC COUNT OF BACTERIA IN MIXTURE OF SEPTIC SEWAGE WITH TEN VOLUMES OF STERILIZED SEPTIC SEWAGE.

Number of hours from beginning of experiment..	0	3	6	24
Bacteria per c.c.....	45,000,000	21,000,000	19,000,000	61,000,000
'Duplicate count).....	54,000,000	17,000,000	15,000,000	39,000,000

The crucial test of the microscopic count could not be made with sewage since with a mixture of various germs the presence of forms incapable of development in our nutrient media excludes the possibility of a control by the plate method. We therefore next examined pure cultures of certain bacteria which grow readily on the gelatin plate. Table 8 shows the result of parallel cultural and microscopic counts of *B. megatherium*; and the agreement is in most cases quite as good as could be obtained between two plate counts of fluids so rich in bacterial life.

TABLE 8.

COMPARISON OF MICROSCOPIC COUNT AND PLATE COUNT.  
*B. MEGATHERIUM.*

NUMBER OF HOURS AFTER BEGINNING OF EXPERIMENT		BACTERIA PER C.C.	
		Microscopic Count	Gelatin Plate
Peptone at 37°	{ 0.....	104,000,000	123,000,000
	{ 17.....	16,400,000	17,200,000
	{ 41.....	144,000,000	134,000,000
	{ 65.....	48,000,000	17,600,000
Broth at 10°	{ 0.....	8,000,000	1,060,000
	{ 53.....	20,000,000	25,800,000
	{ 72.....	26,700,000	34,800,000

In the first experiment of Table 8, there twice occurred a marked falling off in the number of bacteria, 100,000,000 per c.c. disappearing in each case in about 24 hours. With a view to comparing more fully the value of the microscopic count with

increasing and with decreasing numbers of bacteria we examined in parallel, cultures of *B. megatherium* in water at 20° and in Dunham's peptone solution at 27°. In water the bacilli rapidly decreased and in peptone solution they multiplied; in general the agreement of the two methods of counting is very close, only four wide deviations occurring in the whole table, in one of these the gelatin count being in excess. Losses of 200,000 bacteria out of 250,000 and of 112,000,000 out of 116,000,000 in four hours are correctly registered by the microscopic method as well as numerous less marked diminutions.

TABLE 9.

COMPARISON OF MICROSCOPIC COUNT AND PLATE COUNT. *B. MEGATHERIUM* IN WATER AT 20° AND IN PEPTONE SOLUTION AT 37°.

NUMBER OF HOURS AFTER BEGINNING OF EXPERIMENT	BACTERIA PER C.C.			
	IN WATER		IN PEPTONE SOLUTION	
	Microscopic Count	Gelatin Count	Microscopic Count	Gelatin Count
0.....	254,000	157,950	250,000	187,200
48.....	245,000	.....	200,000	283,000
52.....	50,000	.....	950,000	.....
96.....	0	1,000	1,550,000	500,000
119.....	0	0	2,335,000	.....
0.....	1,200,000	.....	1,220,000	1,150,000
1.....	950,000	13,400	1,350,000	1,345,000
22.....	550,000	530,000	1,600,000	1,550,000
43.....	350,000	540,000	4,000,000	4,100,000
0.....	435,000	360,000	435,000	520,000
20.....	.....	8,000	2,195,000	13,764,000
25.....	15,000	2,000	4,030,000	4,590,000
44.....	0	1,840	580,000,000	255,200,000
47.....	6,500	3,060	340,000,000	298,840,000
51.....	.....	.....	600,000,000	126,360,000
0.....	116,000,000	100,000,000	116,000,000	110,000,000
4.....	4,600,000	3,400,000	2,000,000	2,500,000
28.....	.....	.....	34,000,000	29,000,000

Finally we carried out a series of experiments with mixed cultures of two bacteria, *B. subtilis* and *M. roseus*, which could be easily separated both on the plate and under the microscope, in order to study the interaction of two distinct species. Both individual cells and groups of each form are tabulated separately

in Table 10 with gelatin plate counts of both in the last two series. In the first three experiments the colonies of *M. roseus* could not be counted because with inadequate dilutions the bacilli liquified our plates before the cocci developed.

Again the plate counts and the groups noted under the microscope correspond very closely except with regard to the bacilli in the last two experiments which showed low numbers on gelatin. In this particular case it is apparently the plate method which was in error since the steady increase noted under the microscope could only have been conditioned by a corresponding multiplication of living germs. While the bacteria increased, the cocci for which 37° is too high a temperature, showed a general decrease. In the first experiment nearly 2,000,000 disappeared in six hours, in the second, 22 million in two hours; in the fourth 225 million out of 675 million in two hours; in the fifth, 100 million out of 250 million in two hours.

TABLE 10.

COMPARISON OF MICROSCOPIC COUNT AND PLATE COUNT. MIXED CULTURE OF *B. SUBTILIS* AND *M. ROSEUS* IN DILUTED BROTH AT 37°

Number of Hours from Beginning of Experiment	Bacilli per c.c. Microscopic Count	Groups of Bacilli per c.c. Microscopic Count	Bacilli per c.c. Gelatin Plate	Cocci per c.c. Microscopic Count	Groups of Cocci per c.c. Microscopic Count	Cocci per c.c. Gelatin Plate
0.....	2,700,000	.....	4,000,000	1,600,000	.....	.....
6.....	70,800,000	.....	22,000,000	0	.....	.....
0.....	1,200,000	1,200,000	1,000,000	18,900,000	7,800,000	.....
2.....	7,200,000	5,700,000	2,500,000	22,800,000	7,700,000	.....
4.....	50,700,000	26,700,000	30,000,000	0	0	.....
0.....	3,900,000	1,800,000	3,000,000	322,500,000	148,800,000	.....
2.....	3,900,000	2,700,000	5,000,000	344,000,000	137,000,000	.....
6.....	30,400,000	12,000,000	6,000,000	386,800,000	184,400,000	.....
8.....	31,500,000	18,000,000	12,000,000	381,000,000	223,000,000	231,000,000
0.....	1,800,000	1,200,000	690,000	697,000,000	277,000,000	231,000,000
2.....	6,300,000	3,900,000	2,000,000	673,000,000	226,000,000	238,000,000
6.....	45,000,000	13,800,000	670,000	454,000,000	200,000,000	217,000,000
24.....	153,000,000	88,500,000	.....	336,000,000	144,000,000	.....
0.....	5,400,000	3,000,000	80,000	178,200,000	76,000,000	70,000,000
2.....	9,000,000	5,700,000	2,000,000	244,000,000	128,000,000	77,000,000
4.....	16,800,000	9,000,000	2,000,000	154,000,000	91,000,000	77,000,000
6.....	37,000,000	23,000,000	4,000,000	148,000,000	94,000,000	84,000,000
24.....	63,000,000	27,000,000	1,000,000	186,000,000	88,000,000	108,000,000
30.....	151,000,000	72,000,000	.....	126,000,000	73,000,000	133,000,000

Altogether the results of our experiments show that with cultures of germs which flourish on the ordinary nutrient media the

microscopic count and the plate count closely correspond, whether the numbers present be increasing or decreasing. Under the latter condition very large numbers of bacteria, 100,000,000 or more to the c.c. may perish from various natural causes and leave no trace discernible under the microscope. The conclusions drawn from the study of cultures sterilized by artificial procedures which may alter the character of the bacterial cell are therefore not applicable to ordinary conditions. There appears to be no appreciable error due to the presence of dead cells when the microscopic count is applied under varied conditions to organisms which grow on nutrient media; and there is therefore no reason to suppose that such an error will affect its results in the examination of fluids containing organisms of other sorts.

If we are justified in this view the difference between the number of bacteria in sewage, as determined by the plate method and the microscopic count, must depend mainly upon the third of the three factors suggested at the beginning of this communication, the presence of organisms which do not thrive on our ordinary nutrient media. This factor cannot well be measured; but its importance must be great when we remember that some spirilla, the nitrifying organisms and many parasitic bacteria, never appear upon our plates. Heyden's Nährstoff medium, with many substances, shows ten times as many bacteria as the gelatin plate; yet, at least two of the classes above mentioned find this substratum totally unfitted for development. In the microscopic counting of sewage samples many of these forms are at once apparent, spirilla, for example, and very large swollen bacilli, and the groups of variable short rods lying in irregular masses of zoöglöea so characteristic of the nitrifiers. Again, of the bacteria which do grow in gelatin, many, like some of the cocci, develop colonies so slowly that they do not appear for four days or more and are practically never counted in our routine analysis. Even among the ordinary metatrophic species individuals are often present which are too feeble to form colonies on solid media; for example, it is a common phenomenon in water analysis for dextrose broth tubes to yield a positive test for *Bacillus coli*, when agar plates inoculated from the same sample give no colon-like colonies.



Our ordinary quantitative bacteriological methods yield satisfactory comparative results whose value has been proved by long and varied application. The number of colonies appearing on the gelatin plate corresponds pretty closely to the amount of decomposing organic matter in a water, and for ordinary sanitary purposes nothing is likely to supplant this method for quantitative analyses. In many instances, however, a knowledge of the number of bacteria of all types might prove of advantage. How wide the application of such a new method may be can only be determined by the detailed study of various substances in connection with new sanitary problems as they arise. In work on bacteriolysis it offers unique advantages for observing the dissolution of the bacterial cell. In milk analysis it has been suggested that it might find application. Wherever it is desirable to know the grand total of bacteria present we believe that this process deserves recognition. In fluids containing 25,000 or more bacteria to the c.c. enumeration under the microscope is rapid, easy, and accurate; and it measures the absolute number of microorganisms present with far greater precision than any other process.

## REPORT OF THE COMMITTEE ON ANTITOXIC AND IMMUNIZING SERA.

AS THE Laboratory Section of the American Public Health Association stands for uniformity of method in all routine laboratory tests, it seemed desirable to your committee to take up and consider for its present field of work the application of this principle to the routine testing of antitoxins.

It was deemed wisest first to undertake work on the standardization of the method of testing diphtheria antitoxin, leaving those for tetanus antitoxin and other immune sera for such action as their future therapeutic standing would seem to warrant.

As the subject deals with reactions governed by laws which are at present imperfectly understood, and as the results of these reactions are made manifest only through the use of animals, thereby introducing many unknown factors, it is clearly evident to all that any standard test will be a purely arbitrary one, and that the results obtained by its use can be considered only to approximate accuracy and uniformity.

It is not our intention to reconsider or discuss in detail the investigations of Behring, continued by Ehrlich and his followers in their extensive work, which resulted in the formulation of the so-called Ehrlich Standard Unit for diphtheria antitoxin. A brief review of the events leading up to it will suffice.

Prior to 1897 the test generally applied for the determination of the antitoxic strength of sera was based on the formula arbitrarily chosen by Behring to represent the unit for diphtheria antitoxin. This may be defined as follows: An antitoxic unit is 10 times the amount of antitoxin required to leave intact a 250 gram guinea pig after the injection of 10 times the certainly fatal dose of the toxin,\* for pigs of this weight. The method employed was to mix 10 times the minimum dose of a toxin fatal to a 250 gram guinea pig with different quantities of antitoxic serum, and to inject the same subcutaneously into guinea

\*This was the ordinary fresh toxin produced in the Behring Laboratory.

pigs of the required weight. That amount of serum which apparently neutralized the pathogenic action of this amount of toxin contained one-tenth of an antitoxic unit. In other words, 90\* to 100 minimum fatal doses of the usual diphtheria toxin were to be neutralized by one unit of antitoxin.

Ehrlich in 1896 found that this was not always the case. By using various toxins, and the same toxin at different periods, he observed that a unit of a given serum did not neutralize the same number of fatal doses of such toxins but that the latter varied within such wide limits as from 30 to 130 fatal doses. On the other hand he found that the power of a given toxin to combine with an antitoxin remained constant within narrow limits, and he was led to establish a standard antitoxin in place of a standard multiple of the minimum fatal dose of toxin. Such a standard antitoxin is prepared by him under such elaborate precautions as he believes will insure its permanency, and from time to time, at present every two months, portions of the same are sent out dissolved in a mixture of glycerin and salt solution, for the purpose of enabling producers of antitoxin to standardize such toxins as they desire to use in testing the strength of antitoxic sera. One c.c. of a given dilution of this standard serum represents, according to Ehrlich, one unit of diphtheria antitoxin.

This standard unit of antitoxin has been adopted officially by the German government, and has been used as the standard by the official or prominent laboratories in France, Austria, Denmark and England, in Europe, and by the state laboratories in Massachusetts and New York, and the majority, at least, of the private producers of antitoxin in America. Your committee, therefore, will not enter into any discussion or consideration of either the theoretical or practical problems connected with the establishment of this unit, but will pass on to the discussion of the methods for testing the specific strength of diphtheria antitoxins by the use of this standard antitoxic unit or of the Standard Serum issued by the United States Public Health and Marine Hospital Service.

In a general way the procedures are as follows:

\*Some fraction of the 10 fatal doses was neutralized by the guinea pig.

If we have at hand either of these standard sera, it becomes necessary for the standardization of a toxin to add to that amount of a dilution of a standard serum which contains exactly one unit, such an excess of toxin that the resulting mixture will just prove fatal to a standard weight guinea pig on the third or fourth day after its subcutaneous injection. This point Ehrlich denominates L+. The amount of toxin which would be needed to neutralize completely the antitoxin he designates L0. Both these terms are now commonly employed and are recommended to be used in designating these points. Having determined the L+ dose of a given toxin, that amount of any antitoxic serum which when mixed with this dose will prevent the death of a standard weight guinea pig before the fourth day after injection contains one unit of antitoxin.

From time to time members of your committee have conferred with one another concerning the methods used by each one in the application of both the tests for the determination of the L+ dose of toxins by the use of the Standard Serum and the estimation of the antitoxic strengths of sera therefrom. These consultations led to a certain slight degree of uniformity in the methods used, but it has seemed desirable to several members to have the various methods discussed, and one, uniform at least along general lines, adopted as a standard.

One of the members of the committee, having observed that the results of his test on a given toxin did not agree with those of a co-worker on the same toxin, decided to ascertain how far uniform the results obtained by different investigators in different laboratories, and under their varying environment, might be. To this end he requested samples of toxins from seven laboratories, which he tested with the following results:

TOXIN A.—L+ dose said by sender to be 0.32 c.c.

Tested for 0.33 guinea pig died in 27 days.

"	"	.34	"	"	"	"	"
"	"	.35	"	"	"	"	"
"	"	.42	"	"	"	6	"
"	"	.43	"	"	"	4	"

TOXIN B.—L+ dose said by sender to be 0.76 c.c.

Tested for 0.76 guinea pigs all died in 4 days.

TOXIN D.\*—L+ dose said by sender to be 0.33 c.c.

Tested for 0.33 on a large number of guinea pigs at different periods and all died within four days.

\*Toxin C had not been tested by Ehrlich's method.



TOXIN E.—Same as D, with same results.

TOXIN F.—L+ dose said by sender to be 0.19 c.c.

Tested for 0.19 guinea pigs died in 48 hours.

" " 0.185 " " " " 4 days.

" " 0.18 " " " " 4 "

Subsequent to these tests it was deemed advisable for some one member to submit samples of an antitoxic serum to the other members for the purpose of obtaining the results of tests made by each member using the methods regularly employed for this work in his laboratory. These results are presented in the following tables. Each table represents some of the tests applied by one member. With but one exception the method of ascertaining the L+ dose of toxin by the use of the Ehrlich Standard Serum, and of testing the serum against that dose, was employed. In this case the former method of Behring, modified so that 90 minimum lethal doses of a fresh toxin are neutralized by one unit of antitoxin, was employed. The results in those tests were as follows:

TABLE 1.

## BEHRING'S TEST MODIFIED.

Toxin 90 M.L.D.+1	300 c.c.	Serum (300 units per c.c.)	Animal lived.
" " " "	1 325 c.c.	(325 " " " )	" died 4½ days.
" " " "	1 350 c.c.	(350 " " " )	Animal died 4 days.
" " " "	1 350 c.c.	(350 " " " )	Animal died 3½ days.

TABLE 2.

## EHRICH'S METHOD.

DATES	L + TESTS WITH EHRICH'S SERUM		L+ DOSE USED AGAINST SERUM TESTED	NUMBER OF UNITS PER C.C. TESTED FOR					
	For	Result		300	310	320	325	330	350
May 7.....	.23 c.c.	-8*							
	.24 "	-7							
	.24 "	-6							
	.24 "	-5							
	.25 "	-3							
	.25 "	-3							
	.26 "	-3							
July 9. ....	.25 "	+							
	.275 "	+							
July 16 .....	.28 "	-4							
	.3 "	-3							
	.32 "	-2							
Sept. 7.....	.27 "	-3							
	.28 "	-3							
	.29 "	-3							
Sept. 13.....	.26 "	+	.26	+	+	-3	-3	-3	-2

\*The interpretation of the signs in all the tables should be as follows:

+ = Animal lived.

- = " died.

Numerals = Days after injection on which animal died

TABLE 3.  
EHRlich's METHOD.

DATES	TECH- NIQUE	L + TESTED FOR OR USED	NUMBER OF UNITS PER C.C. TESTED FOR				
			262.5	275	287.5	300	350
August 31.....		.53 +					
		.53 +					
		.54 -5					
September 3.....		.54 +					
		.55 -2					
		.55 -2					
September 6.....		.56 -3					
		.56 -2					
		.545 -3					
September 13.....	1	.545 -4					
		.545				++ -4†	-3† -4†
		.545				-3 +	-3 -3
	2	.545				-4† +†	-2† -3†
		.545				-4 +	-2 -2
		.545				-4† -4†	-2† -3†
September 19.....	3	.545					
		.545					
		.545					
September 27.....	1	.545					
		.545					
		.545					
	2*	.545					
		.545					
		.545					
September 27.....	3	.545					
		.545					
		.545					
October 10.....	1	.545					
		.545					
		.545					
October 18.....	3	.545 -3					
		.545 -4					
		.55 -3					
	3	.55 -3					
		.55 -3					
		.55 -3					

Technique No. 1. Ordinary 1 c.c. pipettes and ordinary syringes.

2. Dilutions made in titrated flasks. Ordinary syringes and Ehrlich pipettes used.

3. Ehrlich pipettes and Rosenau injecting syringes used.

\*Slight alterations in technique were made in these tests.

† Dilutions in these tests made at 15° C. All others at 23°-25° C.

TABLE 4.  
EHRlich's METHOD.

DATE	L + TESTS WITH EHRlich's SERUM		L + USED AGAINST SERUM TESTED	NUMBER OF UNITS PER C.C. TESTED FOR		
	For	Result		300	325	350
September 29 .....	.20 c.c.	-2½ ±	.2	-1½	-1½	-1½ ±
	.21	-1½			-1½ ±	

TABLE 5.  
EHRlich's METHOD.

DATES	L+ TESTS WITH EHR- LICH'S SERUM		L+ USED AGAINST SERUM TESTED	NUMBER OF UNITS PER C.C. TESTED FOR					
	For	Result		250	300	312.5	325	337.5	350
May 18 .....	.74	-4							
	.745	-4							
	.75	-3							
May 27 .....	.74	+							
	.745	-5							
June 4 .....	.74	-6							
June 27 .....	.745	-3*	.745*	+	+				
	.745	-6							
July 6 .....	.74	-3	.74				-8		-3
	.74	-5							
July 15 .....	.745	+	.745		+	+	+		
July 26 .....	.745	+							
	.75	+	.75				+	-3	-3
August 25 .....	.74	+							
	.745	-3	.745		+		-3	-3	
	.75	-3							
September 27 ...	.745	-6				+	+		
	.745	-8							

\*The tests for L+ when performed upon the same date as those upon the serum were actually a part of the same series, and the diluted toxin injected was taken from the same cylinders with the same pipette for both the L+ tests and the serum tests, thus controlling the dilution for the L+ with each serum test.

The member making the tests in Table 5 desires to call attention to the fact that when the controls of the L+ dose showed this to be too low, the serum tests showed a corresponding increase in the antitoxic power of the latter. (Tests on July 15 and 26). The errors in these tests must therefore have been in the making of the original toxin dilution if the previous and subsequent tests for the L+ and the antitoxin are to be taken as a standard. On those occasions an unusual number of tests made a slight change of technique necessary.

The results of the tests shown in the various tables may be summarized as follows:

Taking the end reaction in Table 1 as the neutralization of the toxin, as prescribed for that method, the results indicate that the serum contained 300-325 units of antitoxin per c.c.

Taking the end reaction in the tests performed according to the Ehrlich method, as the death of the guinea pig on the fourth day, the results indicate as follows:

Table 2.	310-320	units per c.c.
" 3.	275-287.5	" " "
" 4.	Less than 300	" " "
" 5.	312.5-325	" " "

While these results appear to show a fair degree of uniformity for a series of such tests, an examination of the tables shows that many individual results were quite out of harmony with the average, especially when slight changes in technique occurred. This would be more noticeable if all the tests performed on the serum were included, as was not the case in Tables 5 and 2, where some of the tests for L + were not given.

In the interests of economy of effort and in order to limit the number of tests which need to be performed on any one serum, and yet to attain as great an approach to accuracy and uniformity in results as possible, it has been deemed best by your committee to recommend that the procedures and precautions outlined below be adhered to in the making of tests for the determination of the strength of diphtheria antitoxic sera.

The committee recommend the adoption of the method for such procedures devised by Ehrlich, which, in a general way may be outlined as follows: First, the determination of the amount of diphtheria toxin necessary to kill in four days a guinea pig of approximately 250 grams weight when mixed with one unit of a Standard Antitoxin; second, the determination of that amount of a serum which when mixed with this dose of toxin will prevent the death of a guinea pig of the same weight for four full days. This amount of a serum is to be considered as containing one unit of diphtheria antitoxin.

For the making of dilutions of both toxin or antitoxic sera, including the Standard Serum, the following recommendations are made:

First, that a sterile, .85 per cent solution of sodium chloride (C.P.) in water be used as the diluting medium.

Second, that either sterile glass containers (measuring cylinders and flasks), accurately graduated to contain the desired amount of salt solution or of dilutions be used, or that sterile ungraduated containers to which the salt solution is distributed from accurately



graduated burettes be substituted. Preferably the burettes should be those whose readings have been certified to by either the Prussian or American official testing bureau. (Prussian, Physikalische technische Reichsanstalt. American, Bureau of Standards, Washington).

Third, that for the measurement of the undiluted toxin or serum, including the standard serum sterile capacity bulb pipettes with one mark certified graduations on the stem be used. Such pipettes should be washed out in the solution receiving their contents.

Fourth, that for the measurement of the diluted toxin or serum, sterile bulb outflow pipettes with certified graduations upon the stem be used.

Fifth, that the toxins and serums used be removed from the refrigerator just before their measurement, and that the salt solution be at room temperature.

Sixth, that the total amount of the mixture of toxin and serum dilutions to be injected be as closely approximate to four c.c. as possible.

Concerning the size and number of the dilutions of both toxin and serum, the committee have decided not to recommend any one scheme, believing that on any plan rigidly adhered to, and with the precautions recommended strictly observed, any suitable method should give approximately uniform results.

Several plans are used by the various members of the committee, and an outline of some of these will appear as an appendix to the report.

In injecting the mixture of toxin and antitoxin the following precautions are recommended:

First, that the syringe employed be one that will uniformly deliver its entire contents, including the emptying of the needle. For this purpose it has been found that the syringes of the Koch type are to be preferred to those having pistons. The system worked out by Rosenau<sup>1</sup> of using the barrels of specially devised syringes of the Koch type as mixing chambers for the toxin and serum dilutions fulfils the above requirement, and has the advantage of

<sup>1</sup> *Treas. Dept., Pub. Health and Mar. Hosp. Serv., Hyg. Lab. Bull.* No. 19, October, 1904.

doing away with the extra mixing chamber, and the necessity of drawing the mixture up into a syringe from such a chamber.

Second, that the mixture of toxin and serum dilutions be thorough and complete. To that end, and to permit time for at least the beginning of the union of toxin and antitoxin to take place in vitro, it is recommended that the mixture stand for 15 minutes at room temperature (when not below 15° C.) before injection.

Third, that the injection be made subcutaneously into the subcutaneous tissues of the anterior abdominal wall of the selected guinea pig, the needle being introduced posteriorly and directed toward the median line.

Fourth, that the hair of the animal over the site of penetration be removed before the operation.

In the selection and general treatment of the guinea pigs to be the subject of these tests care should be exercised.

One of the members of the committee<sup>1</sup> has called special attention to the existence of strains which are unusually resistant to the poisonous effects of diphtheria toxin. This insusceptibility is transferred from the female possessing it to her offspring.

In laboratories raising their own guinea pigs care should be exercised in using for breeding purposes females which have survived injections of either toxin or toxin-antitoxin mixtures, inasmuch as their survival may have been due to a special resistance to diphtheria toxin, and this character may be transmitted to their offspring.

The following recommendations are made concerning the selection and treatment of guinea pigs:

First, that only half-grown animals in excellent condition, and born of mothers not known to be unusually resistant to diphtheria toxin be used for these tests.

Second, that the animals shall weigh before feeding on the morning of the day of operation not under 235 or over 275 grammes. Experience has shown that the animals coming within these limits are for all practical purposes evenly susceptible to diphtheria toxin.

<sup>1</sup>THEOBALD SMITH. *Jour. of Med. Research*, 1905, 13, pp. 341, 348.

Third, that after operation the animals shall be kept in cages allowing three-quarters to one square foot of floor space per animal, without handling or disturbance other than what is absolutely necessary for careful daily weighing\* and for feeding them, for four full days. During this time any evidences of a pathological condition which can be observed without disturbance should be recorded.

Fourth, that after this period of four days the animal should be examined and weighed, and any condition of edema, induration, or necrosis at the site of injection recorded.

Fifth, that if the animal dies as a result of the test an autopsy should be performed, and the macroscopic pathological conditions noted. Especial attention should be paid to ascertaining whether the injection had been properly made into the subcutaneous tissues or had been accidentally made into the abdominal cavity.

Your committee believe that an observance of the recommendations herein made will strongly tend towards uniformity of results and ease in the application of the test.

Inasmuch as the committee were without power to add to their number, they desire to state that Dr. M. J. Rosenau, Director of the Hygienic Laboratory, United States Public Health and Marine Hospital Service, was invited to take part in their deliberations and that they are indebted to him for valuable suggestions.

Respectfully submitted,

HERBERT D. PEASE, *Chairman.*

J. J. KINYOUN.

JOSEPH MCFARLAND.

WM. H. PARK.

THEOBALD SMITH.

#### APPENDIX.

The choice of a system of diluting both the toxins and the sera in the determination of L+ dose, and in the regular tests for antitoxic strength, will depend on one's choice in the method of making the final injection of the mixtures. The methods in vogue for the latter are of two types: first, those that aim so to arrange the systems of dilution as to give approximately two

\* Any handling of animals after injection may affect them injuriously and some members do not recommend the daily weighing. Any course decided upon should be adhered to.

c.c. each of the toxin and serum dilutions, and secondly, those that make up the final total of four c.c. by the addition of salt solution either to the final mixture or by using the additional salt solution for washing the last slight traces of the mixture in the syringe into the guinea pig.

If the first plan is chosen, then it is necessary to arrange the dilutions of the toxin so that the dose to be injected will be about two c.c. For example, if the L+ of a given toxin was suspected, or had been determined to be .21 c.c., it would require a dilution of 10 times in order to give that amount of toxin in approximately two c.c. (actually 2.1 c.c.) of the dilution.

For the dilution of the Standard Serum, the dilution given on the bottle as necessary to obtain one unit in one c.c. would have to be doubled in order to obtain one unit in two c.c. For example, where the bottle label gives one c.c. of serum mixture plus 12.25 c.c. of diluent as a dilution containing one unit per c.c., it would be necessary to make a dilution of one c.c. of serum plus 25.5 c.c. of diluent in order to obtain one unit in two c.c.

For the dilution of the sera to be tested, all that is necessary under this system is to make dilutions such that the denominator of the fraction, which represents the amount of serum, in two c.c. of dilution, is the same as the number of units per c.c. suspected of being contained in the serum to be tested.

Thus, if a given serum is to be tested for a possible strength of 300 units per c.c., a dilution of 1 c.c. serum + 99 c.c. salt solution, and of this 2 c.c. + 10 c.c. of salt solution will give  $\frac{2}{100}$  c.c. of the original serum in 2 c.c. of dilution. By this system the addition or subtraction of 1 c.c. to or from the 10 c.c. of salt solution in the second dilution will raise or lower the units to be tested for by 25.

Where the other type of system is used, any or even no dilution of the toxin, and any dilution of the serum, giving in the amount injected that fraction of one c.c. of serum, the denominator of which represents the number of units to be tested for, will be sufficient for the performance of the test, and will only require the addition of the salt solution to make the total mixture four c.c. For example:

Toxin L+ = .21 .21 measured directly by pipette into cylinder holding final mixture or diluted 1 c.c. toxin + 4 c.c. salt sol. = .21 c.c. in 1.05 c.c. dilution.

Serum to be tested for 300 units per c.c.:

1 c.c. serum + 19 c.c. salt sol.

1 c.c. of first dilution + 14 c.c. salt sol. =  $\frac{1}{100}$  c.c. in 1 c.c. of second dilution.

Or another method:

1 c.c. serum + 9 c.c. salt sol.

1 c.c. of first dilution + 29 c.c. salt sol. =  $\frac{1}{300}$  c.c. in 1 c.c. of second dilution.

The opportunities for error in measurement are, of course, the least in the greater dilution.



## THE IMPORTANCE OF THE PARADYSENTERY BACILLI.

WM. H. PARK,

Professor of Bacteriology and Hygiene in the University and Bellevue  
Hospital Medical College.

THE investigations upon the bacteriology of dysentery in the United States have revealed the fact that in many of the sporadic cases and in quite a few epidemics none of the bacilli described by Shiga have been present. In cases occurring in the Middle Atlantic and New England states there have been found chiefly two types of bacilli which though resembling the Shiga bacilli in many respects yet differ from them in others of great importance, viz.: in specific agglutinins and immune bodies and in the formation of indol and in the fermentation of mannite in peptone solution. In this medium one type also actively ferments maltose and saccharose, while the other type does so very feebly, if at all. In a few sporadic cases, several different varieties of bacilli varying from these types have been the only organisms found. The prevalence of the cases due to these bacilli is underestimated, since many of them have been reported, as if due to the Shiga bacillus. This is because the cultures were not at first carefully studied, or because the name of Shiga was given out of compliment to the one who, in establishing the bacillary origin of dysentery in temperate climates, opened the way to the discovery of the bacilli differing from the type discovered by him. Few realize that the great majority of the cases of dysentery in Baltimore, as well as those in New York, Boston and Philadelphia have not revealed the Shiga bacillus, but have revealed the bacilli of the types designated by us as the paradysentery bacilli.

It seems to us that the statement of Hiss,<sup>1</sup> that further investigation is necessary to establish the claim that these paradysentery bacilli are inciters of disease is too conservative. In the article by Drs. Collins,<sup>2</sup> Goodwin, and myself we collected a number of sporadic and epidemic cases of dysentery in which

<sup>1</sup> *Jour. of Med. Research*, 1904, 13, p. 49.

<sup>2</sup> *Ibid.*, 11, p. 553.

paradysentery bacilli were the only organisms of the dysentery type present.

These bacilli were present alone in so many cases that the proof that they were inciters of the disease seemed as conclusively shown as in the cases where the Shiga bacillus was found.

During the summer of 1904 we investigated a number of cases of dysentery in New York City and they gave the same findings as those of the years previously reported. Only a small percentage of the cases contained Shiga bacilli, all the others containing one or another of the paradysentery varieties. On Staten Island, which is situated about six miles from New York City, in the bay there was a severe localized epidemic of dysentery, from which all the cases examined gave the Shiga bacillus.

An epidemic at a dumping station was so striking in that the people were isolated from those outside that, although previously published, it is here repeated.

At Rikers' Island a number of men were filling in new land. The privy arrangements were very poor, and infection readily took place. Dysentery broke out and spread to a number of the men, as well as to the physician in charge. Those infected had usually a short, sharp attack with a quick recovery. Very large amounts of blood were passed by some of the sick. In some a large proportion of the bacteria isolated were bacilli of the Manila type. (Flexner). No other type of dysentery bacilli was found in any of the cases in this epidemic.

One of last summer's cases gave very interesting findings as seen in the following account:

Girl, 8 years. Had an acute attack of dysentery with tenesmus, abdominal pain and frequent passages. Pronounced symptoms lasted eight days. On the third day a stool was put into four ounces of sterile water and sent directly to the laboratory. When received there was a little blood and a moderate amount of mucus present with the fecal matter. A series of plates was made by Dr. Mary E. Goodwin from a piece of bloody mucus. 69 colonies were fished from them at the end of 20 hours. Of these 69 colonies 50 were formed of bacilli of one of the mannite fermenting paradysentery types. The organism isolated agglutinated as follows in the patient's blood:

	1-100	1-200	1-400
Organism isolated	++	±	—
Paradysentery b. Mt. Desert type (Park)	++	+	—
Paradysentery b. Manila type (Flexner)	+	—	—
Dysentery b. (Shiga)	—	—	—

The agglutination of the Manila type in 1:100 dilutions was due to common agglutinins and is of almost constant occurrence in the blood of those suffering from colon or dysentery bacillus infections. When the culture was placed in a serum which after purification by extraction of agglutinins contained specific agglutinins for the Manila type only no agglutination took place at any dilution, while in a serum containing specific agglutinins for the Mt. Desert type it agglutinated completely, but, as in the case of the patient's serum in slightly lower dilutions than the laboratory culture of the Mt. Desert type, which had been kept on agar for three years.

In this case where the examinations were made by Dr. Goodwin we have characteristic symptoms, the presence during the disease of abundant bacilli of a single paradysentery type and no bacilli of any other dysentery or paradysentery type, the development of specific agglutinins for that type and for no other and the disappearance of the bacilli with the cessation of the symptoms.

The causal relationship between this bacillus and the case is to my mind as well proven as in any case of dysentery in which the Shiga bacillus was found in the bloody mucus and specific agglutinins in the blood.

The mannite fermenting types are widely scattered over the world, and to me they seem to be inciters of characteristic cases and epidemics of dysentery, although on the average the disease caused by them is milder than when due to the Shiga bacillus. One or the other of these types also appear at times in small numbers in mixed infections where dysenteric symptoms are almost or entirely absent.

It seems to me convenient and proper to restrict the name dysentery to bacilli having the characteristics of the bacillus first identified by Shiga, and give the name paradysentery to the other varieties of bacilli which approach more closely the colon group in that they produce indol and have a greater range of activity in fermenting carbohydrates. While it seems wise to separate the dysentery and paradysentery bacilli, the name dysentery remains a purely clinical term and includes under it cases excited by both varieties of bacilli as well as by protozoa.

## THE OCCURRENCE OF BACTERIUM PNEUMONIAE IN THE SALIVA OF HEALTHY INDIVIDUALS.\*

W. D. FROST, C. B. DIVINE AND C. W. REINEKING.

(From the Bacteriological Laboratories of the University of Wisconsin.)

### INTRODUCTION.

It is generally recognized that *Bacterium pneumoniae*† is found in the saliva of a considerable proportion of healthy individuals. The statement is frequently made that it occurs in one out of every five persons. But the experiments upon which these conclusions are based have been made by different observers at various times of the year and under a variety of conditions; so much so that it seemed worth while to restudy this question and to determine independently the distribution of this germ in the saliva. The salivas of some 50 individuals have been examined, and in some cases that of the same individuals at different times of the year. This was done to determine whether or not there was any variation in the seasonal distribution of the germ. The results seem to show that there is marked variation in the distribution of this germ at different seasons of the year. Other examinations have been made upon different classes of individuals at the same season of the year to note if any variation occurred which could be properly assigned to differences of occupation. Here again a variation appeared, especially when the distribution of the germ in the saliva of those employed "indoors" was compared with the distribution of the germ in the saliva of those employed "out of doors." The authors are well aware that the conclusions which might be drawn from these experiments are not conclusive, but it is believed that the results obtained are of sufficient import to warrant a somewhat detailed account of the experiments and the data collected.

\*The work here described formed the basis for theses which were presented for the bachelor's degree at the University of Wisconsin, by Mr. (now Dr.) Divine in 1900 and by Mr. Reineking in 1904.

†Throughout this paper the term *Bacterium pneumoniae* is used instead of the more common term of "pneumococcus." This is in accordance with Migula's system of classification, which seems to be the one most widely accepted.



## METHODS EMPLOYED.

The presence of *Bacterium pneumoniae* has been determined exclusively by the inoculation of rabbits with the saliva. The inoculations have been made as soon as possible after collection. The rabbits received approximately two c.c. intraperitoneally. In cases where the animals died the autopsies were performed as soon as possible. Cover-slips of the blood from the various organs were stained by means of the Welch capsule stain. Cultures were also made from the various organs. At first these were made on blood agar prepared according to the method recommended by Eyre and Washburn.<sup>1</sup> In this case the identification of *Bacterium pneumoniae* was confirmed by the appearance on this agar of small pinhead colonies. Later cultures were made directly into milk. In this medium the organism in question produces a thick curd and cover-slip preparations therefrom show beautiful capsules with the capsule stain. Those cases only were counted as positive in which there developed a septicemia and in which there was found in the blood of the rabbit, or milk subcultures therefrom, a lancet shaped diplobacillus and on which a definitely stained capsule could be demonstrated. In a few cases in order to economize on rabbits the saliva of several individuals, which seemed unlikely to contain the germ, were mixed and the mixture inoculated into a single rabbit. In case the rabbit died it was necessary to retest the saliva of each individual separately; but if the animal remained well it was taken for granted that none of the group contained the organism in question.

## EXPERIMENTAL WORK.

From Table 1, which gives the details of the work, it is seen that there have been 85 inoculations. Thirty-two of these gave a positive result, or 37.6 per cent. The saliva was obtained from 50 different individuals. Eighteen of these showed the presence of this germ, or 36 per cent. The percentage of positive results obtained is higher than that reported by previous observers.

Of the 50 individuals tested 26 were university students, 2 were high school children, 6 were ward school children, 5 were teamsters, 5 were outside laborers (carpenters), 5 were draftsmen and 1 was a housewife. All were well at the time the saliva was collected and it is not known that any of them fell sick for some-time afterward, so that they may fairly be considered to have been healthy at the time their saliva was examined.

## SEASONAL DISTRIBUTION.

A considerable part of the inoculations were undertaken, as already indicated, for the purpose of determining whether or not

TABLE 1.  
INOCULATION OF RABBITS WITH THE SALIVA OF HEALTHY INDIVIDUALS.

NO. OF EXPERI- MENT	INDI- VIDUAL	TIME OF INOCULA- TION	RESULT		NO. OF EXPERI- MENT	INDI- VIDUAL	TIME OF INOCULA- TION	RESULT	
			Neg.	Pos.				Neg.	Pos.
1.....	A1	Oct.-Nov.	0		44.....	C5	Jan.-Feb.	0	
2.....	A2	"	0		45.....	D1	"		+
3.....	A3	"		+	46.....	D2	"	0	
4.....	A4	"	0		47.....	D3	"	0	
5A.....	A5	"	0		48.....	E1	"	0	
6.....	B1	"	0		49.....	E2	"		+
7.....	B2	"		+	50.....	E3	"	0	
8.....	B3	"	0		51.....	E4	"	0	
9.....	B4	"		+	52.....	E5	"		+
10.....	C1	"	0		53.....	F1	"	0	
11.....	C2	"	0		54.....	F2	"	0	
12.....	C3	"	0		55.....	F3	"	0	
13.....	C4	"	0		56.....	F4	"	0	
14.....	C5	"	0		57.....	G1	"		+
15.....	D1	"	0		58.....	G2	"	0	
16.....	D2	"	0		59.....	G3	"		+
17.....	D3	"	0		60.....	G4	"		+
18.....	E1	"	0		61.....	H1	"		+
19.....	E2	"	0		62.....	H2	"		+
20.....	E3	"	0		63.....	H3	"		+
21.....	E4	"	0		64.....	H4	"	0	
22.....	E5	"	0		65.....	H5	"	0	
23.....	F1	"	0		66.....	I1	Mch.-Apr.		+
24.....	F2	"	0		67.....	I2	"	0	
25.....	F3	"		+	68.....	I3	"	0	
26.....	F4	"		+	69.....	I4	"		+
27.....	G1	"		+	70.....	I5	"	0	
28.....	G2	"	0		71.....	J1	"	0	
29.....	G3	"		+	72.....	J2	"		+
30.....	G4	"	0		73.....	J3	"		+
31.....	A1	Jan.-Feb.		+	74.....	J4	"	0	
32.....	A2	"		+	75.....	J5	"		+
33.....	A3	"		+	76.....	K1	"	0	
34.....	A4	"	0		77.....	K2	"	0	
35.....	A5	"	0		78.....	K3	"	0	
36.....	B1	"		+	79.....	K4	"	0	
37.....	B2	"	0		80.....	K5	"	0	
38.....	B3	"		+	81.....	L1	"		+
39.....	B4	"		+	82.....	L2	"		+
40.....	C1	"	0		83.....	L3	"		+
41.....	C2	"	0		84.....	L4	"		+
42.....	C3	"	0		85.....	L5	"		+
43.....	C4	"	0						

the season of the year had any influence on the prevalence of the germ in the saliva. On account of the large number of rabbits required periods were selected about two months apart. In the year 1900 the saliva of 30 individuals was examined in the fall and then again in the winter months. The figures given for the spring months were obtained in 1904 and from an entirely different set of people. While the results obtained then in the first and second periods are directly comparable, the results obtained for the spring months can not be so closely compared with the preceding. The results obtained show that of the 30 examina-

tions made in the fall 7 were positive, or approximately 24 per cent; that of the 35 inoculations made in the winter 15, or 43 per cent, were positive; and that of the 20 inoculations in the spring 10, or 50 per cent, were positive.

#### VIRULENCE OF THE GERM.

Another question of considerable importance is that of the virulence of the germ of pneumonia as it occurs in the saliva. This has been judged entirely by the period elapsing between the time of inoculation and the death of the rabbits. This time has varied from 20 to 100 hours. The details are represented graphically in Fig. 1.

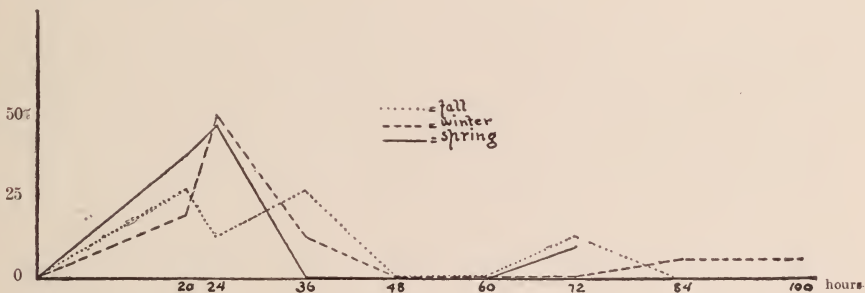


FIG. 1.—Virulence of *Bacterium pneumoniae* as indicated by the course of the disease in rabbits. The ordinates represent the per cent of rabbits which died at or within a given time. The abscissae are the intervals expressed in hours.

It would thus appear that the virulence of this germ was greater during the time when it was most prevalent and lower when it was less common. In other words it appears that 90 per cent of the rabbits died within 24 hours in the spring, about 73 per cent in the winter, and only about 42 per cent in the fall months.

#### PERSISTENCE OF THE GERM IN THE SALIVA.

Still another point of some interest is the question as to whether or not the germ persists in the same throat for sometime. The condition of affairs as it appeared in this work is shown in the following table:

TABLE 2.  
PERSISTENCE OF THE PNEUMONIA GERM IN THE SAME THROAT AT DIFFERENT SEASONS  
OF THE YEAR.

	GROUP														
	A1	A2	A3	B1	B2	B3	B4	D1	E2	E5	F3	F4	G1	G3	G4
Fall .....	-	-	+	-	+	-	+	-	-	-	+	+	+	+	-
Winter .....	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+

It is seen here that of the 7 individuals whose saliva contained *Bacterium pneumoniae* in the fall only 4 contained it in the winter months, and that of the 12 individuals who harbored it in the winter 8 had acquired it since the previous examination. Or again only 4 out of the 15 gave the same reaction at both examinations.

#### INFLUENCE OF OCCUPATION.

In regard to the influence of occupation on the presence of this germ in saliva but little can be determined from the earlier experiments (Nos. 1 to 60) since they were not performed with this idea in view and all of the subjects were selected from practically the same occupation; but the latter experiments (Nos. 61 to 85) bear directly on this subject. In this part of the work three classes were studied: those who were indoors almost entirely, those in and out, and those who worked outside all day. For convenience they were divided into groups. Group L consisted of five draftsmen, working in a machine shop. Groups H and I were five university students. The group designated J were teamsters or hack drivers who worked outside a great deal but not constantly. The last group (K) was composed of five laborers who worked out of doors all day all of the year around as carpenters.

TABLE 3.  
INFLUENCE OF OCCUPATION ON THE DISTRIBUTION OF THE PNEUMONIA GERM IN SALIVA.

Group	No. of Indi- viduals Tested	Occupation	No. of Indi- viduals Reacting	Per Cent Reacting
K.....	5	Laborers	0	0
J.....	5	Teamsters	2	40
I.....	5	Students	2	40
H.....	5	Students	3	60
L.....	5	Draftsmen	5	100



These results suggest that occupation may be a factor in accounting for the variation of the distribution of this germ in the saliva of healthy individuals.

#### SUMMARY.

Eighty-five rabbits have been inoculated with saliva causing sputum septicemia in 32 or 37.6 per cent.

The saliva of 50 different individuals has been examined and in 18 or 36 per cent of the cases *Bacterium pneumoniae* has been found.

The frequency with which this germ appears seems to vary with the season of the year, 23 per cent of the salivas examined in the fall being positive, 43 per cent in the winter and 50 per cent in the spring.

The virulence\* of the germ, as indicated by the rapidity of the course of the disease in the rabbit, varies, the greatest virulence appearing during the period of greatest frequency.

The germ may persist in the saliva of certain individuals from season to season but more than 66 per cent of the cases positive in winter were negative in the fall.

*Bacterium pneumoniae* appears to be more frequent in the saliva of individuals working "indoors" than those working "outdoors."

\*No account is taken of the number of germs that might be present.

## LABORATORY METHODS AND DEVICES.

F. F. WESBROOK,

Director of Minnesota State Board of Health Laboratory.

*(From the Minnesota State Board of Health Laboratory and the Department of Pathology and Bacteriology, University of Minnesota.)\**

### APPARATUS FOR THE COUNTING OF COLONIES IN PETRI DISHES.

*(Designed March, 1904, and Described by Louis Blanchard Wilson.)*

The apparatus consists essentially of a dish holder bearing a circular glass plate appropriately ruled with circular and radiating lines. By means of a three-jawed chuck acting like an iris diaphragm, the Petri dish by a single motion of a lever is accurately centered over the ruled plate and securely held in place. In this position colonies in cultures which for any reason may not be inverted (e. g. much liquefied gelatin cultures) are counted. But preparations which may be inverted (e. g. all agar cultures and all gelatin cultures only slightly liquefied) are readily turned over by rotating the holder, thus bringing the ruled plate above the bottom of the inverted dish and placing the preparation in the most advantageous position for counting.

Under the dish holder is placed an adjustable swinging plane mirror of larger diameter than the dish. The obverse side of the mirror is dead black.

Above the holder is a swinging arm for holding a lens of any desired (low) magnification. The lens, by means of joints and a revolving sleeve, may be focused readily on any portion of the culture. (The designer prefers a large, low-power  $2\frac{1}{2}$  diameters "reading glass" for routine work.)

The above parts are supported on a heavy rod rising from an ample horseshoe base. With the exception of the glass portions the apparatus is constructed entirely of brass, polished and lacquered, and is thus durable and neat. It is made by Mr.

\*Some of these methods were presented before the Laboratory Section of the American Public Health Association at Buffalo in 1901, but no published description appeared from either of the laboratories. They were therefore included in the demonstration before the Section at the Havana meeting, 1905, and embody suggestions and devices originating with different members of the two staffs and are here presented by the Director of the laboratories.

L. U. Boyle, Mechanician, Medical Laboratories, University of Minnesota, who also has solved most of the mechanical problems involved, though valuable suggestions have been received from Drs. M. Russell Wilcox and E. H. Beckman.

A photograph of the apparatus is shown in Fig. 1.

The advantages of the apparatus are as follows:

1. The Petri dish culture may be instantly changed from the erect to the inverted position or to any inclined angle without changing the relative position of the ruled glass plate, the ruled side of which is held constantly in apposition to the bottom of the dish.

2. The dish is always accurately centered over the ruled plate. This greatly diminishes the error in estimates made from alternate counted segments of preparations in which the colonies are too numerous to permit of all being counted.

3. Every advantage of light is afforded by the large swinging mirror with its reflecting and dead-black surfaces.

4. The lens holder is readily adjustable and permits the use of various lenses, to which may be attached a *camera lucida*.

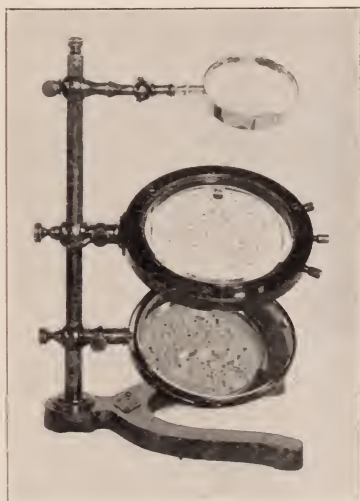


FIG. 1.

#### STANDS FOR MUSEUM EXHIBITION OF PERMANENT FIXED CULTURES OF BACTERIA.

These stands are constructed and the parts assembled by Mr. L. U. Boyle. The stands themselves, the component parts, and the methods are illustrated by the accompanying photograph (Fig. 2). The wooden frame is made of closely grained wood, suitably stained, and consists of a heavy doubly beveled base with two pillars, across the top of which is a flat support, perforated. The cultures are arrested at a suitable stage of their

development by fixation with formalin. The cotton plugs are withdrawn from the mouths of ordinary test tubes in which the cultures are grown. The test tubes are placed in large air-tight jars and formalin poured into the bottom of the jar or upon cotton. The formation of formic acid may be prevented by the addition of ordinary chalk. For gelatin stab cultures or deep

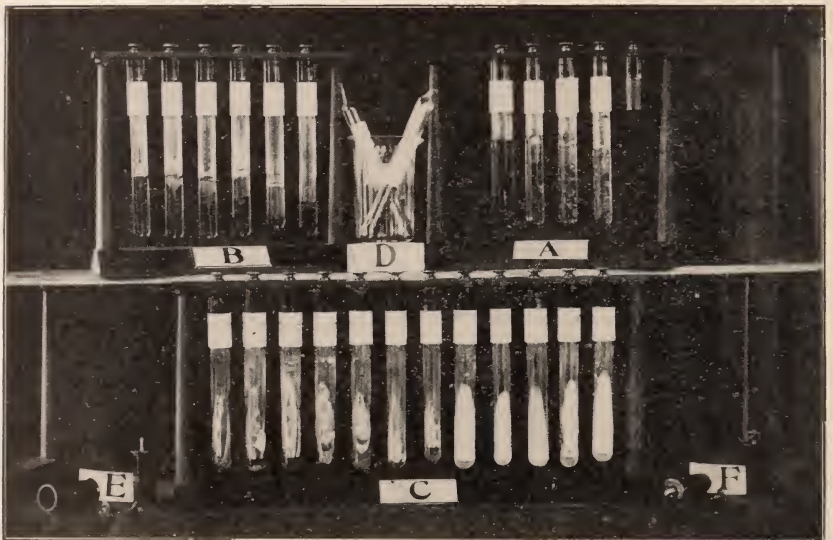


FIG. 2.

cultures, such as anaërobes, in which it is imperative to stop immediately the growth of the culture in the depth of the medium, the formalin may be added by a small glass pipette blown from ordinary glass tubing. The pipette filled with formalin is thrust to the bottom of the culture tube along one side, and as it is withdrawn the formalin is deposited in the pipette track.

When properly fixed, the test tubes are sealed in the blow-pipe flame, and in such a way as to leave a knob or a hook on the top. The hermetical sealing prevents evaporation (D, Fig. 2). The caps for these tubes are made from ordinary gun shells, which can be obtained of various gauges. Those having high brass bases are preferable. The shells are cut off and the cap



extracted. The percussion cap is replaced by a threaded brass pin, driven firmly into position. The nuts are made with milled heads. The preserved, sealed cultures are fixed in the cartridges with moist plaster of Paris. The threaded stem is passed through the perforation in the wooden support and the nut screws the tube in position. Labels may be attached to both the tubes and the stand and coated either with shellac or paraffin.

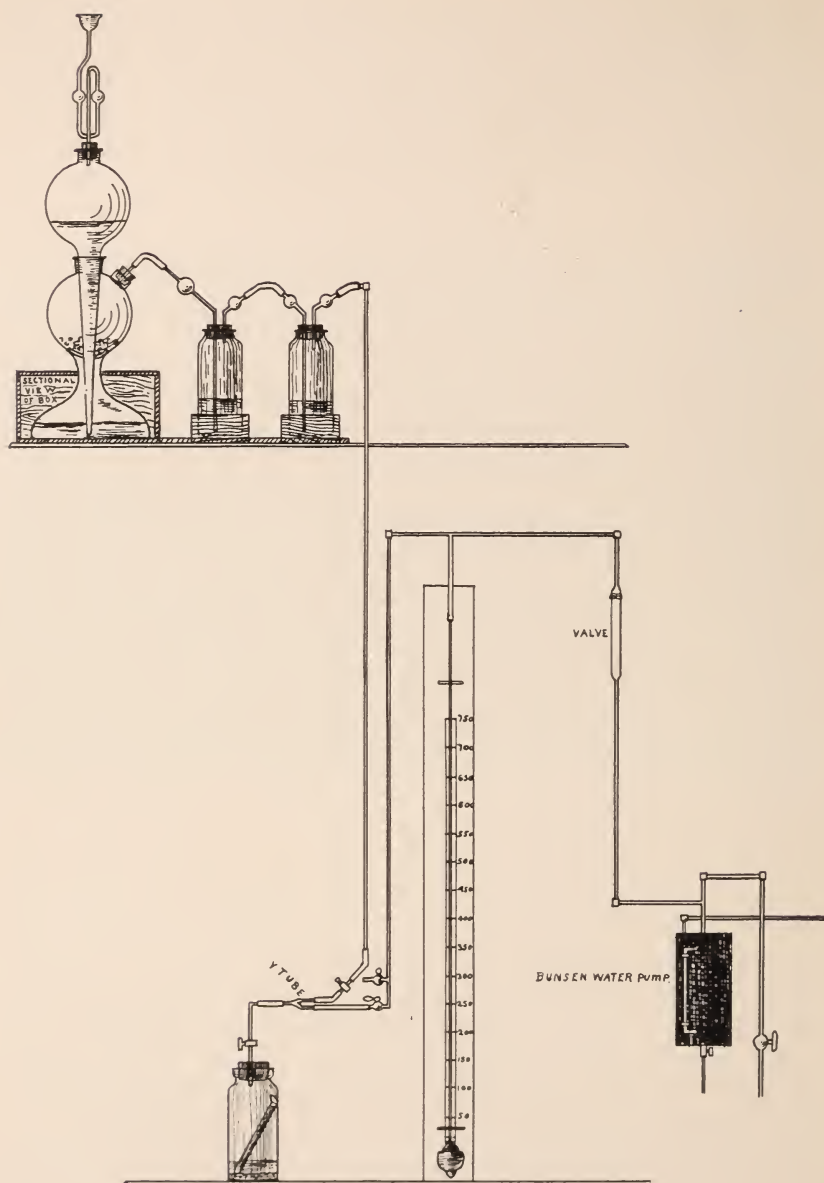
The special advantages of this method are,

1. The entire tube and its contents are shown. The ordinary test tube rack obscures the bottom of the tube.
2. Facility in grouping the cultures is afforded.
3. The permanency of all cultures, except chromogens which are affected by the formalin, is assured, since evaporation is prevented.

#### COMPLETE AND RAPID ANAEROBIOSIS.

Fig. 3 illustrates the combination of methods which is employed. A Kipp's hydrogen generator and two wash bottles for cleaning the gas are placed on a shelf in the neighborhood of a sink. A Lautenschläger or other suitable modification of the Bunsen pump, is placed in position over a sink and brass or other metal pipe connects the water pump with a mercury or other manometer and is finally connected with the Kipp's apparatus by means of a Y tube. The third limb of the Y is connected with a jar or sectional receptacle designed by Novy.

For the inoculation of agar, gelatin or broth tubes, the medium is boiled immediately before use and inoculated with the microorganisms which it is desired to observe either after the medium has been set or, in the case of "shake" cultures, before solidification occurs. A wide-mouthed bottle has a layer of sand in the bottom. A solution of pyrogallic acid is poured upon the sand. The tubes are placed in position in the bottle. The wide-mouthed bottle is provided with a rubber stopper having one perforation. In the perforation a bent glass tube with a ground gas-tight, freshly vaselined cock, is fitted. The rubber stopper is coated with paraffin so as to make a gas-tight joint with the neck of the bottle and to prevent the adherence of the rubber to the glass. Just prior to the insertion of the stopper into the



bottle a small stick of caustic soda or potash is dropped into the pyrogallic solution. The water pump is used for exhausting the air in the bottles and the cock in the tube leading from the Kipp's apparatus is turned off, both cocks between the pump and the bottle being turned on. When exhaustion is complete, the tap between the Y tube and the pump is turned off and the tap on the tube leading from the Kipp's apparatus turned on very gradually in order that hydrogen may replace the air which has been exhausted from the bottle. Care should be taken not to permit the hydrogen to escape so fast that air is drawn in through the top of the Kipp's apparatus. When the bottle is full of hydrogen, the tap leading from the hydrogen generator is closed and the bottle is again exhausted of its mixture of air and hydrogen. When this is complete, the tap leading to the pump is again closed and more hydrogen admitted. This process is repeated five or six times or oftener, if desired, until all of the air has been completely washed from the bottle, when suction should be applied until a negative pressure of 100 to 150 millimeters of mercury is recorded. The cock in the bent tube in the rubber stopper is now closed and the bottle disconnected from the Y tube. With its contained cultures the bottle is placed in the incubator. The reason for the negative pressure is that it obviates the danger of the expulsion of the stopper when the cool bottle and its contents are placed in the incubator and the contained gas expands. For plates the large sectional receptacle designed by Novy for anaërobic cultures may be used in the same way by replacing the complicated glass stopper with a perforated rubber stopper with bent glass tube and stop cock.

The special advantages of the apparatus and method are the following:

1. No table space is permanently occupied.
2. If gas-tight joints and cocks are provided the apparatus is always ready for use and it is almost as simple to grow anaërobic as aërobic.
3. By firmly fixing the Kipp's apparatus and the two wash bottles to a board the whole apparatus may be taken down very easily for cleaning.

4. If exhaustion of the air follows immediately after the addition of the sodium hydrate the fluid in the bottle remains colorless and this lack of color together with the maintenance of a slight negative pressure, may be taken as the index of complete anaërobiosis.

5. There is a saving of hydrogen as compared with Botkin's or Novy's method.

6. The complete anaërobiosis secured is desirable in testing unknown microorganisms and in the elimination of aërobes during the isolation and identification of bacteria in mixtures.

7. The use of complicated, expensive and fragile apparatus is rendered unnecessary.

#### BI-METALLIC THERMO-REGULATOR WITH SPECIAL ARRANGEMENT FOR EASY CLEANING AND ADJUSTMENT.

Mr. L. U. Boyle, the laboratory mechanician, is the designer and maker of a thermo-regulator which is very like the French pattern. Without a series of complicated sketches or photographs it would be impossible to illustrate it.

The main features are the provision of small metal union joints whereby it is possible to attach a lead or other metal tube directly to the gas supply, to the thermo-regulator, and from the thermo-regulator to the safety lamp. These unions provide a means of easily disconnecting the thermo-regulator or the safety burner from its metal connections. Metal connections for incubators are universally employed throughout these laboratories as being much safer than rubber. Bi-metallic thermo-regulators, with the special union joints, render manipulations as easy as with rubber tubes and protect against the danger of fire.

#### DEVICES FOR DISPLAYING AND LABELING PATHOLOGICAL SPECIMENS.

Fig. 4 shows several features which have been found of great practical use in the pathological museum:

1. Labeling specimens in Whitall-Tatum jars. A satisfactory method of labeling was found to be difficult without obscuring the specimens. The objection to the attachment of the label holder or label to the clamp or top of the jar is that it is apt



to become detached from the specimen or jar to which it rightfully belongs. Thin sheet brass or copper is obtained already cut to size. Along one side and both ends the metal is bent so as to form a groove into which a label card slips. In the center of the



FIG. 4.

sheet, by means of a punch, an opening which is somewhat T-shaped is made so that the metal is severed except at the base. This metal is bent backwards, first at right angles to the plane of the sheet, then again at right angles so that what was formerly the top of the T is now in a plane parallel to the body of the label holder. Through each end of this a hole is punched and annealed copper or brass wire is passed through the holes. This is then applied to the neck of the jar. By means of pliers, or the fingers, the wire is tightly twisted so as to support the label holder in position. A shows the front view of the label holder.

*B* shows the back view with the copper wire in place. *C* shows the label holder containing the label in position.

2. In order to avoid the possible return of a specimen to the wrong jar when once it has been removed, all specimens when received in the laboratory are provided with an accession number. A small tag, *D*, made of stalbite or wood fiber, such as that used by electricians for insulating purposes, is numbered with a die, and through a hole which has been previously drilled is sewn with stout silk into the specimen before it is immersed in the preserving fluid.

3. Other specimen jars. The "Coplin" jar sold by Queen & Co. is shown in *E*. This was found to be somewhat expensive, and the method of fixation of the label in the split ends of the rods which project above the specimen jar was unsatisfactory. In the workshop of the laboratory, patterns were made and the brass rods and bases were cast in a local foundry. Flat specimen jars were imported and solid India rubber gaskets obtained from a local firm. *F* shows the rods, *G* shows the top, and *H* the base of the jar. *K* shows the jar which has been made and assembled in our laboratory workshop. *L* shows a label holder somewhat like that already described, but without the central punch hole, which has been soldered to the brass top. This method of preserving has been found to be very satisfactory, and a jar such as shown in *K* is more easily manipulated and cheaper than the "Coplin" jar, and does not require so much perpendicular space for display. It is unnecessary also to detach the label in opening the jar.

#### AGAR HANGING DROP.

In 1896 a method was devised which would render it possible to study the development of a bacillus from a spore, or vice versa, and which would permit of special observation on the arrangement of bacteria, i. e., their relation to each other.

A coverslip is placed in coverslip forceps and sterilized by passage through the flame. A tube of melted agar is allowed to cool to about 50° C. With a platinum inoculator a minute amount of the microorganism to be observed is streaked in the center of the sterilized coverslip, and by means of a loop a large drop of

the molten agar is placed immediately over the streak. The coverslip is inverted over a vaselined hollow ground slide, and the development of the microorganism, which is now on the top of the flat plane of agar immediately underlying the coverslip, may be followed with an oil immersion lens. This method has been employed for teaching and research purposes for nine years in these laboratories. As an aid to the identification of *B. anthracis* it has proved of great value on more than one occasion.

Hill, who was not aware of the existence of this method, described a somewhat similar procedure, "the hanging block," in 1902.<sup>1</sup>

#### DEVICES FOR STAINING A LARGE SERIES OF MICROSCOPIC CULTURE PREPARATIONS ON COVERSGLIPS OR ON SLIDES.

Two methods have been employed for large series of diphtheria examinations in the State Board of Health routine diagnostic work, where it is necessary to examine and report upon from 50 to 250 specimens in a day.

1. Until within the past year coverslip preparations have been made and mounted with balsam. The tubes as they are received from various localities are numbered serially, and the data slips which accompany them are correspondingly numbered. They are then placed in tumblers numbered 0 to 9. After being incubated overnight, the tumblers are placed in regular sequence in a row. Two kinds of coverslip forceps are used and grouped in sets of 10. The individual forceps in each set are numbered 0 to 9. When Cornet coverslip forceps are used the numbers are struck upon the upper surface with a metal die. When Stewart coverslips forceps are used a small disc of copper numbered with a metal die is slipped into place and soldered in the loop in the middle of the upper surface. By the use of these numbered coverslip forceps, the relationship of the coverslip preparation to the corresponding tube can be maintained without danger of confusion until the specimen is finally mounted on a labeled slide.

2. Recently, staining upon the slide has been employed, and in some respects is more convenient, although not so cleanly, nor are the mounts so permanent.

<sup>1</sup> *Jour. Med. Research*, 1902, 2, p. 202.

Fig. 5 shows a device first suggested by Dr. L. B. Wilson and improved by Drs. Beckman and McDaniel. It is constructed of metal and does not bend appreciably when heated. The slides are numbered with a glass pencil, and the preparations as made are placed in regular sequence in the apparatus, which consists essentially of a tray without a bottom other than the two strips of metal placed on edge. After the preparations are dried, the tray is placed crosswise over a sink and an ordinary Bunsen burner held in the hand of the operator is used for flaming—the depth of the sink permitting of the application of the flame to the under surface of the slides. Löffler's methylene blue or other stain is poured upon the upper surface of the slides and, if desired, heat



FIG. 5.

may be applied either to the upper surface or from underneath. Distilled or tap water by means of a rubber tube with a glass tip is used for washing the microscopic preparations without disturbing them in the tray. The whole tray is picked up, allowed to drain somewhat, then placed between two folds of smooth filter paper and blotted on both sides. It is convenient to place the tray upon a piece of blotting paper which is more than double the width of the tray; the edge of the blotting paper is then folded over the top of the tray and the slides are firmly pressed and rubbed with the hands through the blotting paper. By inverting the tray, still wrapped in the paper, all the slides are turned out upon the paper and the tray removed. The paper is again folded in position so as to be on both sides of the slides. It is now reinverted so as to leave the slides in regular sequence face up. When completely dried they are ready for examination, and the sequence has been maintained.



## "WIDAL" REACTION IN TYPHOID FEVER.

An envelope is furnished to physicians which contains the following named articles:

1. A piece of aluminum wire, No. 19 gauge, 7.5 cm. long, bent at one end into a loop 0.3 cm. in diameter.
2. A strip of aluminum foil, No. 40 gauge, 5 cm. square.
3. A data blank as follows:

MINNESOTA STATE BOARD OF HEALTH LABORATORY  
(University of Minnesota), Minneapolis.

DATA TO ACCOMPANY SPECIMEN FOR TYPHOID FEVER EXAMINATION.

Date and hour of collection.....

Patient's Name.....Address.....

Physician's Name.....Address.....

Health Officer's Name.....Address.....

Has this case been reported upon before?.... If so, give previous case No.....

Patient's Age..... Sex..... Temperature.....

How long since disease commenced?.....

What is the supposed source of infection?.....

When, if ever before, has the patient had Typhoid Fever?.....

Remarks.....

.....

Physician's diagnosis..... Do you desire a telegraphic report?.....

The envelope has printed on it the following:

MINNESOTA STATE BOARD OF HEALTH LABORATORY  
(University of Minnesota), Minneapolis.

OUTFIT FOR COLLECTING SPECIMEN OF BLOOD FOR SERUM DIAGNOSIS OF TYPHOID FEVER.

To secure a reliable reaction with dried blood it is necessary that a comparatively large amount be collected in as cleanly a manner as possible. Hence please observe carefully the following directions:

Wash with boiled water the part from which the blood is to be obtained (the lobe of the ear, end of finger, or toe in infant). Prick deeply the skin with a clean needle or scalpel. Remove four or five loopfuls of blood with the wire loop in outfit, placing each by itself near one edge of the aluminum square enclosed. Make a roll about 1 cm. in diameter—of the square, turning inward the blood without smearing it. Flatten one end of the roll and turn the edge over to prevent it from opening. *Allow the blood to dry*, then make a tight packet of the roll by flattening and turning over the other end. Fill out the data blank *in full*, return it with the foil packet and wire-loop to its envelope; place this in a larger envelope and mail to the Laboratory.

On receipt in the laboratory the flat packets are numbered with a pencil or blunt point. When they are opened the dried blood readily flakes from the foil. A portion is removed with a

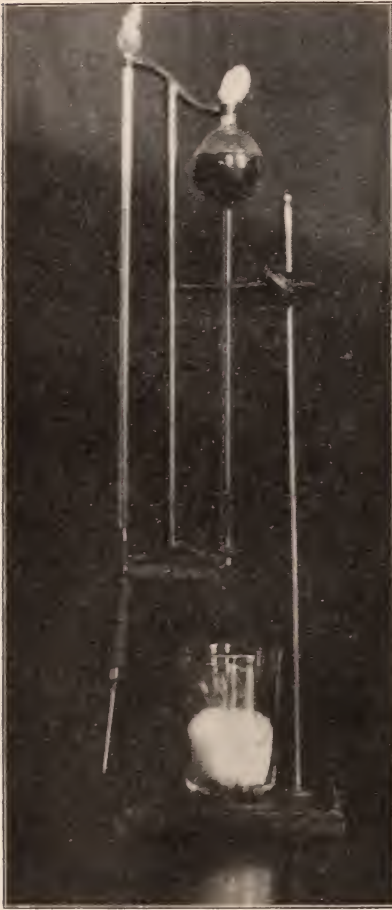


FIG. 6.

spatula, and 0.001 gramme is weighed on a fine balance. Two small squares of aluminum foil with rounded corners bent so as to form a shallow trough are kept on hand. They are of exactly the same weight, and used, one in each scale pan. One is used as a counterpoise. The small pieces of blood are dropped upon the other to be weighed and by means of a clean camel's hair brush, when weighed, are brushed into a small test tube. To the blood in the test tube, by means of a special pipette (Fig. 6), 0.1 c.c. of distilled water is added. This gives a dilution of 1:100 of the dried blood or 1:25 of the fluid blood. After shaking, the tube is allowed to stand for 30 minutes. From the supernatant fluid, by means of a large platinum loop kept for this purpose alone, a drop is placed upon a clean coverslip and by means of a very small platinum loop the drop is inoculated from a fresh broth

culture of *B. typhosus*. The loops are kept for this purpose alone and the very great difference in size renders the error in dilution practically negligible. At the end of two hours the presence or absence of the reaction is recorded.

The details of the collection, transmission and weighing of the

blood were elaborated by Dr. Louis B. Wilson and the method partially described in the *Philadelphia Medical Journal*, March 26, 1898.

The pipette shown in Fig. 6 is made by fusing a "T" tube into the end of an ordinary one c.c. water pipette graduated in hundredths. The bulb for containing the diluent is blown on an ordinary glass tube approximately of the same size as the pipette and bent as shown in the plate. The tube leading from the bulb is connected with the T piece of the pipette by a rubber tube and a glass bead is used in lieu of a pinch cock. This is also used between the end of the pipette and the dropper. The pipette may be filled to any desired point by means of the "bead-cock" and the desired amount allowed to escape from the pipette through the dropper into the test tube containing the dried blood. Care should be taken that there are no bubbles in the rubber tubes and that the dropper is absolutely filled with fluid. The metal frame, made in the laboratory workshop, holds the apparatus and renders it possible when the pipette and bulb are filled with fluid to sterilize the whole outfit in the autoclave. This combination of methods permits of accuracy and does not interfere with the operation of the laboratory over a wide territory since the drying of the blood permits of no changes in transit.

#### METHOD OF PREPARING RAPIDLY A SERUM FOR DIPHTHERIA DIAGNOSIS, WHICH WILL YIELD A MEDIUM OF CONSTANT QUALITY.

Large quantities of ox serum are collected at the slaughter house, brought to the laboratory and stored in tightly stoppered bottles with an excess of chloroform (approximately one per cent). These are kept cool and in the dark until a sufficient quantity of serum is accumulated.

The different lots of serum are blended in a large receptacle. To three parts of the serum is added one part of a one per cent glucose peptonized broth which contains five per cent glycerin.

This blended mixture, of sufficient quantity to provide for several months or a year, is bottled and tightly corked after chloroform in excess has been added to each bottle. It is then kept cool and in the dark until required for use.

When required the mixture is tubed and the tubes placed in a copper "sloper." Both front and back views of the "sloper" are shown in the accompanying photographs (Fig. 7). This "sloper" is made throughout of copper in order to facilitate rapid and even



FIG. 7.

transmission of the heat. For convenience in filling, the "sloper" is tilted against the wall. It is so arranged that when it rests in its normal upright position, the requisite amount of slope is given to the serum in the tubes.

When all the tubes have been filled and placed in position, the "sloper" is set in an autoclave (Wiesnegg) which is *very gradually* heated with pin-point gas flame, the steam cock remaining open. If the flame is properly adjusted, at the end of



about one and one-half to two hours, steam begins to escape through the steam cock. The steam cock is closed 15 minutes after the steam has begun to escape and the flame under the autoclave is turned somewhat higher until at the end of a half hour a temperature of 120° C. is attained. This is maintained for 30 minutes when the flame is turned out. In the hands of the attendant, one heating yields serum ready for use which is smooth and free from bubbles. The blending of a year's stock gives a serum of practically constant composition for that period. The use of glycerin, which was begun early in our work with a view to preventing evaporation, has been continued, since all of our observations in relation to the diphtheria bacillus for the past seven years have been based upon cultures grown on this medium.

#### A MODIFIED DRIGALSKI-CONRADI CRYSTAL VIOLET MEDIUM.

This medium is a very stiff nutrient agar to which is added litmus solution and a solution of crystal violet. The method of preparation as modified by Dr. E. H. Beckman and used in the Minnesota State Board of Health Laboratory is as follows:

(a) *Nutrient agar*.—Add one liter of water to 680 grammes of finely-chopped lean beef and place in the cold for 24 hours. Express the juice and make up to one liter. Coagulate the albumin either by vigorous boiling for 10 minutes or by heating at 120°C. in the autoclave. Filter. Add 10 grammes of Witte's peptone, 10 grammes of nutrose, and 5 grammes of sodium chloride. Heat in the autoclave at a temperature of 120°C. for 30 minutes or boil vigorously for 15 minutes. Render slightly alkaline to litmus paper. Filter. Add 30 grammes of agar. Heat in the autoclave at a temperature of 120°C. for one-half hour or heat over the gas flame until the agar is dissolved. Render slightly alkaline to litmus paper while hot, if necessary. Filter through glass wool into a sterile vessel.

(b) To 130 c.c. of litmus solution (Kubel and Tiemann's) add 15 grammes of chemically pure lactose. Boil for 10 minutes.

(c) Mix (a) and (b) while hot. Render slightly alkaline to litmus paper, if necessary.

To the mixture add two c.c. of hot sterile solution of 10 per cent sodium hydrate in distilled water and 10 c.c. of a fresh solution of Höchst's crystal violet (0.1 gramme of crystal violet to 100 c.c. of sterile water).

The medium is now poured into Petri dishes and is of a deep purple color. So much water of condensation forms on the solidified surface that it is an advantage to use porous clay covers (Hill) for the Petri dishes instead of the ordinary glass cover. The medium keeps well but dries up rapidly.

After the medium has stood for 24 hours, it affords a smooth, firm surface upon which bacteria may be sown. The glass rod described in the original article<sup>1</sup> seems to be the best instrument for streaking out cultures.

The advantages of the medium are as follows:

1. The medium is not easily contaminated.
2. The acid colonies are easily distinguished.
3. Many of the common varieties of bacteria such as staphylococci and streptococci grow very slowly, if at all.
4. *B. typhosus* and *B. coli communis* grow rapidly and abundantly and the colonies of each can readily be distinguished. The larger size of the typhoid colonies, as stated in the original article,<sup>1</sup> has not been observed in this laboratory.
5. *B. typhosus* and *B. coli communis* can be obtained easily and rapidly in purity from mixed cultures.
6. All the colonies are on the surface and can be seen and transplanted with ease.

#### TESTS OF VALUE OF EMBALMING FLUIDS.

The Minnesota State Funeral Directors Association has had this matter under consideration for a number of years. The Association appointed a committee to investigate the efficiency of ordinary commercial embalming fluids with a view to determining and publishing suitable formulae for embalming purposes. An embalming fluid to be satisfactory should render inert all pathogenic bacteria in the cadaver without interference with the "cosmetic" effect which is so desirable in their work.

The Minnesota State Board of Health was requested to undertake the necessary laboratory investigation and the following

<sup>1</sup> *Ztschr. f. Hyg. u. Infektionskrankh.*, 1902, 39, p. 291.

methods are in the process of test and further elaboration by Dr. R. H. Mullin.

1. Test of germicidal efficiency. A mass culture of *B. typhosus* is made in sterile blood serum, pleuritic or other highly albuminous fluid comparable in nature to the body proteid content. Such culture after incubation for 24 hours is distributed in test tubes in 15 c.c. amounts. To each tube is added 0.8 c.c. of one of the embalming fluids. (The respective amounts above stated are employed because in embalming as ordinarily done, four quarts or eight pounds of embalming fluid is the maximum amount used for each 150 pound cadaver, in arterial injection.) After 24 hours, from each of the test tubes including controls, sub-cultures are made and results recorded. Those fluids which show germicidal properties according to the foregoing test are further investigated as follows:

2. Test of embalming qualities and "cosmetic" effects are made by the Embalmers' committee upon human cadavers.

3. Test of germicidal properties in embalming human cadavers may be used but more exact observations are possible by the use of rabbits which have succumbed to an inoculation of virulent anthrax bacilli so that every tissue and organ is filled with the living bacilli. The rabbits are weighed. Into each, through the left femoral artery, the embalming fluid under observation is injected in the proportion of 8 to 150 body weight. The injected animal is kept at ordinary temperature and at the end of 24 hours sowings are made from definite selected sites which are constant throughout the whole series of experiments. Those which are employed are brain, lung, liver, spleen, right gastrocnemius and contents of duodenum and of the ileum near the valve. Absolutely the same methods in operation and record are employed.

#### FIELD METHODS IN THE BACTERIOLOGY OF WATER.

In order to render it possible to begin the bacteriological examinations of water in the field so as to obviate changes during transmission to the laboratory, the following method has been evolved:

A supply of boxes is maintained for the transmission by express of the requisite materials to the site of investigation. Some of the details of the construction of the box and of its contents ready

for use together with the materials carried by the investigator into the field, may be seen from the accompanying photograph (Fig. 8). These boxes are made of pine, painted on the outside and loosely lined at top and bottom with corrugated paper. They are divided into three compartments by two upright partitions which slide into grooves. One end compartment contains Petri dishes which are wrapped in filter paper and sterilized. The paper in which they are sterilized protects them from breakage during transmission. The central compartment which is narrower than those at the ends, provides for two mushroom ground stoppered bottles of approximately 200 c.c. capacity. A small piece of twine is placed between the stoppers and the inside of the neck of the bottles. Clean muslin, in double or triple thickness, is placed over the stopper and tied in position with twine around the necks of the bottles; these are then sterilized in the autoclave. The twine between the stopper and the neck prevents the impaction of the stopper. In the other compartment, test tubes stoppered with cotton and containing plain agar and litmus lactose agar are carried. A case constructed of thin brass tubing with a slip cover is used to contain three water pipettes which are wrapped in thin paper in the brass case, the cover placed in position and the case and contents sterilized by heat. The hinges and hasps of the box are fastened in position with rivets and the box is so constructed that without mutilation, its contents cannot be disturbed by any one who does not possess a key to the padlock. All of the padlocks for these boxes and the larger boxes used for chemical samples, open by the same key.

The investigator carries with him a gasoline blast lamp (Turner's), a copper boiler which is carried in a leather slip case, a thermometer in a brass case (similar to that used for the pipettes), labels, glass pencils, etc.

Immediately after the collection of the samples, measured amounts are plated with plain and litmus lactose agar. The blast lamp is used for melting the tubes of agar in the copper boiler. After the plates are cool and firmly set they are re-wrapped in filter paper, inverted and packed in the box.

The colon bacillus determination is effected by adding one c.c.



of water to one c.c. double strength agar in the test tube. This agar thus diluted is allowed to set in the test tubes. The used test tubes are again replaced in their compartments, the box properly repacked, locked and shipped by express to the laboratory.



FIG. 8.

On arrival in the laboratory, the plain agar plates are placed at a temperature of  $21^{\circ}\text{C}$ . until ready for count and the litmus lactose agar plates are put immediately into the incubator at  $37^{\circ}\text{C}$ . The test tube containing the solidified agar sown with one c.c. of the water for the colon determination, is placed in the incubator after 10 c.c. of broth has been added and mixed with the agar which is thoroughly broken up with a stiff platinum inoculator. After 24 hours incubation, Smith tubes containing dextrose broth

and carbolized dextrose broth are inoculated from the test tubes containing the mixed agar and broth. From the Smith tubes colon bacillus may be isolated, if present, by streaking or plating. Other amounts of water may be similarly employed if desired. It is impossible by this method to use gelatin plates.

The particular advantages of the method are safety in shipment and protection against artificial contamination or loss of *B. coli communis* when present originally. The agar and litmus lactose agar plates having been inoculated with the water immediately after collection give a definite idea of the numbers and approximately, of the species of bacteria present in the water at the time of collection. These methods are employed in all the Minnesota work which is being carried on jointly by the United States Geological Survey and the State Board of Health and have been thoroughly tested by R. B. Dole, Assistant Engineer, U. S. Geological Survey and Dr. E. H. Beckman, Assistant Bacteriologist, Minnesota State Board of Health.

#### FILING METHODS FOR WATER DATA.

At the meeting of the Laboratory Section in Havana, sample files of the information collected in the joint work of the U. S. Geological Survey and the Minnesota State Board of Health were shown. It is impossible to reproduce the blanks and information at this time. The method consists in filing alphabetically according to locality, typewritten records of visits of inspection, which are supplemented by official statements on suitable blanks from health officers, city engineers, superintendents of waterworks, ice dealers, manufacturers, and others. Blue prints, drawings, photographs and other data illustrative of waterworks, sewerage, sewage disposal works, ice fields, possible sources of contamination, structural features and data concerning geological and meteorological features are filed together with the chemical, bacteriological and biological analyses. It is hoped in this way that the State Board of Health will ultimately have in its possession more complete information concerning each locality than will be possible, in most instances, for the locality itself to obtain and preserve.

## AN IMPROVEMENT IN THE TECHNIC OF THE INDOL TEST.

JOSEPH MCFARLAND AND J. HAMILTON SMALL.

*(The Medico-Chirurgical College of Philadelphia.)*

SOME years ago Dr. Dunham introduced the peptone solution to facilitate the detection of indol, recommending it on the ground that its freedom from color made it more easily possible to detect the reddish tinge of the nitroso-indol than an amber colored fluid such as broth. It was, however, later shown by Theobald Smith that peptone solution is less well adapted to purposes of culture than broth, and after a careful study of the subject Smith recommended that the peptone solution be abandoned for the use of a sugar free broth.

It is customary to test for indol by the addition of a small quantity of a weak solution of potassium nitrite (0.01 per cent solution) and some chemically pure sulphuric acid, the liquid being shaken and the presence of a red color, in case very little is formed, noticed on the whitish froth. When the quantity of indol present is very small, a considerable delicacy of color perception is required to recognize it, so that any method becomes welcome that will concentrate the color at some particular portion of the tube. It has, therefore, occurred to us to endeavor to modify the test by the formation of a color ring, a slight modification of the usual method sufficing for this purpose.

The culture to be tested receives an addition of one drop of chemically pure sulphuric acid for each c.c. of fluid, this being well shaken. In case the microorganisms produce both indol and nitrites the red color now makes its appearance, as in the cholera spirilla, etc., but when the organisms produce no nitrites, as in the case of those of the colon group, after the sulphuric acid has been mixed with the fluid the dilute solution of potassium nitrite is allowed to trickle slowly down the side of the tube and form a layer on the surface of the fluid it already contains. The red color of the nitroso-indol now makes its appearance at the line of

contact of the two fluids where it is quite easy to recognize amounts of indol that could with difficulty be recognized should the tube be shaken and the color diffused through it.

By making solutions of indol crystals in distilled water and testing by this method, we have found it possible to recognize the presence of indol in dilutions of upwards of 1:750,000. In dilutions of 1:1,000,000 the color was not distinctly appreciable. In all lower dilutions the color was in proportion to the intensity of the solution.

Dr. Peckham made use of known dilutions of indol for determining the probable percentage of indol in different cultures, but the color being diffused throughout the entire liquid, the more delicate tints were lost.

It is quite easy to prepare a series of such color rings as have been described for making quantitative comparisons for ordinary laboratory work. We have found it quite satisfactory to make a series of test color rings in solutions containing two per cent of gelatin, the melted gelatin containing the indol and sulphuric acid is placed in a test tube and the melted gelatin containing the dilute nitrite solution is superimposed upon it. The color forms at the line of contact, as usual, and as the gelatin rapidly solidifies such tubes are not disturbed by handling or oversetting and can be kept for comparison for from 12 to 24 hours. Beyond 24 hours, the color begins to diffuse itself through the gelatin and is gradually lost.



COPPER SULPHATE AS A GERMICIDE:  
SOME NOTES ON ITS USE IN CONNECTION WITH SEWAGE  
EFFLUENTS.

GEORGE A. JOHNSON AND WILLIAM R. COPELAND,  
Columbus, Ohio.

COPPER sulphate is a powerful germicide. Canning industries have taken advantage of this fact for a number of years, adding this chemical to meats and vegetables to prevent their fermentation by bacteria and other organisms. More recently copper sulphate has been used to destroy and prevent growths of algae in reservoirs; moreover, sanitarians are devoting considerable attention at the present time to the feasibility of using it to destroy bacterial life in polluted waters.

Following this general line tests are being carried on by the writers to determine the effect of copper sulphate when applied to effluents of sewage purification works. In this paper, certain of the results obtained to date, are presented. A more complete account of this work will be published in the near future.

METHOD OF CONDUCTING THE TESTS.

The experiments were conducted in 100 c.c. volumes, collected from the effluents of several different processes of sewage purification. The typhoid culture used was obtained from Parke, Davis and Company. The culture media employed were prepared in strict accordance with the recommendations of the Committee on Standard Methods of the American Public Health Association.

GERMICIDAL EFFECT PRODUCED BY DIFFERENT CONCENTRATIONS  
OF COPPER SULPHATE.

In testing the germicidal power of different concentrations of copper sulphate, a solution of the commercial article was added in the proportion of one part of the chemical to 200,000, 100,000, 50,000 and 25,000 parts, respectively, of sewage effluents. To some of the samples large numbers of typhoid bacilli were added, before introducing the chemical.

The results presented in Table 1 show that the bacteriocidal power of the copper sulphate increased with the concentration of the solution. It is to be noted, however, that complete sterilization was not effected in 24 hours in any of the samples.

TABLE 1.  
GERMICIDAL EFFECT OF VARIOUS CONCENTRATIONS OF COPPER SULPHATE.

PARTS OF COPPER SULPHATE TO PARTS OF SEWAGE EFFLUENT	BACTERIA PER C.C.		
	Before Adding Copper Sulphate	3 Hours After Adding Copper Sulphate	24 Hours After Adding Copper Sulphate
1 to 200,000 .....	1,400,000*	120,000	1,100
1 to 100,000 .....	1,400,000*	28,000	650
1 to 50,000 .....	1,400,000*	20,000	600
1 to 100,000 .....	240,000§	150,000	800
1 to 50,000 .....	240,000§	84,000	250
1 to 25,000 .....	210,000§	33,000	220

\* Average of four sets of results.

§ Average of three sets of results.

#### RELATION BETWEEN THE PERIOD OF CONTACT AND THE GERMICIDAL ACTION OF COPPER SULPHATE.

As will be seen from the figures given in Table 2, the numbers of bacteria diminished very rapidly during the first hour of contact. The stronger concentrations destroyed the organisms somewhat more rapidly than the weaker.

TABLE 2.  
RAPIDITY OF THE GERMICIDAL ACTION OF COPPER SULPHATE.

BEFORE ADDING COPPER SULPHATE	BACTERIA PER C.C.				
	15 Minutes	30 Minutes	1 Hour	3 Hours	15 Hours
910,000* .....	460,000	270,000	240,000	80,000	700
880,000§ .....	250,000	220,000	190,000	47,000	500

\* In this set the proportion of copper sulphate to effluent was 1 to 100,000.

§ In this set the proportion of copper sulphate to effluent was 1 to 50,000.

Typhoid bacilli were added to these samples before they were submitted to the test, the number of this species largely predominating. In each case the results given are averages of seven sets of experiments.

## EFFECT OF TEMPERATURE ON THE RAPIDITY OF THE GERMICIDAL ACTION OF COPPER SULPHATE.

As is well known, chemical reagents exercise their characteristic properties to a much greater degree at some temperatures than at others. Copper sulphate, for instance, is more active as a germicide at temperatures of about 20° C. than at materially lower temperatures. The effluents of sewage disposal works will vary widely in temperature throughout the year. In order to obtain information on this point, one-half of a series of samples was kept at a temperature of 20° C., and the other half of the series at about 5° C. Copper sulphate was added in different concentrations, and tests for the numbers of bacteria were made at intervals.

The results of these tests are given in Table 3, and show clearly that the bacteria disappeared more rapidly at the higher temperature. Further, it is worthy of note, that at the higher temperatures, particularly in the case of the lower concentrations, the power of the germicide appears to have become exhausted soon after the sixth hour of contact.

TABLE 3.

EFFECT OF TEMPERATURE ON THE GERMICIDAL POWER OF COPPER SULPHATE.

TEMPERATURE DEGREES C.	PARTS OF COPPER SULPHATE TO PARTS OF SEWAGE EFFLUENT	BEFORE ADDING COPPER SULPHATE	BACTERIA PER C.C. AFTER STANDING IN CONTACT WITH COPPER SULPHATE FOR			
			30 Minutes	2 Hours	6 Hours	18 Hours
5	1 to 100,000	460,000	100,000	51,000	24,000	2,600
20	1 to 100,000	600,000	44,000	3,300	700	81,000
5	1 to 50,000	520,000	48,000	34,000	3,900	1,800
20	1 to 50,000	580,000	18,000	2,900	650	3,900
5	1 to 25,000	490,000	37,000	24,000	5,500	1,300
20	1 to 25,000	580,000	15,000	1,300	250	350

No typhoid bacilli were added to these samples. The figures given are in each case the average of three sets of experiments.

## EFFECT OF ORGANIC MATTER, AND OF DISSOLVED CARBONATES OF LIME AND MAGNESIA, ON THE GERMICIDAL POWER OF COPPER SULPHATE.

Effluents of sewage purification works contain more or less organic matter, which probably affects the germicidal power of

copper sulphate to some extent, through absorption. The carbonates of lime and magnesia, dissolved in some sewage effluents, probably affect a considerable precipitation of the copper as a basic carbonate, in which state it is less efficient as a germicide. While the results presented in Table 4 do not show the individual effect of these factors, it is nevertheless evident that the germicidal power of the copper sulphate was less active in the samples of sewage effluent containing organic matter and dissolved carbonates, than in redistilled water which was free from both.

TABLE 4.

EFFECT OF ORGANIC MATTER AND DISSOLVED CARBONATES ON THE GERMICIDAL POWER OF COPPER SULPHATE.

CHARACTER OF SAMPLE	TEMPERATURE DEGREES C.	PARTS PER MILLION		BACTERIA PER C.C.				
		Organic Nitrogen	Dissolved Carbonates	Before Adding Copper Sulphate	After Standing in Contact with Copper Sulphate for			
					5 Min.	1 Hour	3 Hrs.	15 Hrs.
Sewage effluent.	20	2.87	233	1,300,000	830,000	260,000	21,000	600
Distilled water..	20	0	0	1,300,000	420,000	19,000	650	11

Large numbers of typhoid bacilli were added to these samples. The copper sulphate was used in the proportion of 1 part to 50,000 parts of the samples.

#### OTHER DISTURBING FACTORS.

In the consideration of the efficiency of copper sulphate as a germicide in connection with sewage effluents there are other disturbing factors which must be borne in mind, as: for instance, sulphides which, by precipitating the copper, would probably diminish its germicidal power.

#### REGARDING COSTS.

The figures given in the preceding tables indicate that there are a number of important factors which must be taken into consideration in connection with the use of copper sulphate as a germicide. They affect its efficiency, and point to the fact that the numbers of bacteria are likely to diminish so slowly where only one part of copper sulphate is used in 100,000, that the germicide must be added in greater quantities to effect a sterilization of the sewage effluents within a reasonable period of time.



If it shall be subsequently proved that it is necessary to use materially higher concentrations of this chemical than 1 to 100,000, the question of the cost of the treatment will be brought into special prominence.

At the present time the cost of copper sulphate is in the neighborhood of \$120 per ton. Assuming that this figure is correct, the cost for chemical alone is shown in the following table:

COST OF COPPER SULPHATE.

Concentration	Grains per Gallon	Pounds per Million Gallons	Cost for Chemical per Million Gallons at \$0.06 per Pound
1 to 100,000 .....	.585	83.6	\$ 5.02
1 to 50,000 .....	1.17	167.2	10.04
1 to 25,000 .....	2.34	334.4	20.08

# METAMORPHOSIS OF FILARIA IN THE BODY OF THE MOSQUITO (CULEX PIPIENS).

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## INTRODUCTION.

THE names of Demarquay, Lewis, Manson, Cobbold, Low and James may be said to epitomize the history of filaria. They may be divided into two groups; one representing the fortunate discoverers of these curious nematodes, and the other, including Manson, Low and James, the students of the transformations of the parasite in the mosquito.

If we consider that filaria, as demonstrated by Manson, is transmitted by the mosquito, we are disposed to conclude that the prevention of its propagation must be an easy matter; and, theoretically, it is so, provided we can secure a strict isolation of infected patients from the bite of mosquitoes.

There are still some points that require further elucidation, such as the mode of transmission from the mosquito to man, but, from the sanitary viewpoint, it is sufficient to know that the evolution of the filaria embryo occurs exclusively in the mosquito. This knowledge has not been applied in sanitary practice.

My own investigations have concerned themselves with the evolution of the parasite in the body of the mosquito, and with the determination of how, where, and when the worm leaves the body of the insect. My aim has been to include the whole process, and to leave no doubt as to the inoculation of filaria by mosquitoes in the human subject.

## SOURCE OF MATERIAL.

The case utilized for my experiments was one of moderate infection. He was sent to me by Dr. Enrique Nuñez and he subjected himself patiently to many annoying procedures.

The clinical history is briefly as follows:

He had always enjoyed good health, except that he was operated on in June 1903, by Dr. Nuñez, for a left inguinal hernia.

He remembers to have had, since he was eight years old, a slight enlargement of the glands in the right groin. This increased very gradually, extending downwards into the thigh.

Recently the diagnosis of adenolymphocele was made, and the tumor was totally extirpated in January, 1904.

In July, an abscess, about the size of a hen's egg, formed in the left thigh. This was opened, and healed in about nine days. An examination of the blood made at this time revealed a considerable number of filaria embryos.

On the day of admission in "Las Animas" hospital, August 22, 1904, the blood was examined at 8:30 P. M. with negative results. On the following day, at 9:30 P. M. from two to three embryos were found in each preparation. A little later we could find from 10 to 12 in each preparation. We may look upon this as the average during the nights when mosquitoes have been applied.

#### MOSQUITOES, AND TECHNIQUE OF THEIR INFECTION WITH FILARIAE.

Mosquitoes were applied to our patient on seven occasions, separated by the intervals of time necessary for the study of the infected insects. The conclusions of the present paper are founded on the first six applications. The seventh series is still under observation.

The facilities for this line of experiments at "Las Animas" Hospital are great, since we have rooms well screened with wire gauze, and assistants who have been well trained in mosquito work.\*

Several kinds of mosquitoes have been used in these experiments, but the one in which we have been able to follow methodically the phases of development of filaria has been *Culex pipiens*. This particular species must be allowed to bite in condition of relative freedom, under a mosquito net. Our *Anopheles (albipes)*, *Stegomyia*, and *Culex consobrinus* on the other hand, will bite readily in jars. The insects were made to bite at night, in accordance with the law of periodicity that causes the *filaria nocturna* to appear in the peripheral circulation only at night.

In order to obtain infection of the insects by the greatest number of embryos possible, it is advisable to have the patient go to bed, at least two hours before the mosquitoes are let loose under the net. Our failure to comply with this requirement caused a marked diminution of the infection in one of our series.

After remaining under the net all night with the patient, the insects were collected on the following morning.

\*I have to thank Mr. E. Gros, the assistant in the laboratory, for valuable aid in carrying out these investigations.

The hour of 12 P. M. was fixed upon as the time of beginning of the infection. By that time all the insects had surely bitten.

The mosquitoes employed were mostly specimens reared in captivity. Some insects were captured in the attendants' dormitories, where there was no filariasis. There can be no doubt, therefore, that the mosquitoes had not been previously infected.

A curious difference is noticeable between the insects proceeding from these two sources. The insects reared in our breeding jars are much less likely to bite than those captured in adult life. In the former class I have had only two out of 50 insects bite on some occasions; whereas hungry veterans captured in adult life are always successful biters.

Of 11 insects of the species *Culex consobrinus*, that had bitten the patient, not one became infected.

Out of 19 *stegomyias*, only one showed some infection, in the shape of two filariae that were still alive on the fifth day, but poorly developed.

Out of 70 specimens of *Culex pipiens* only eight failed to become infected, and these had been applied under the unfavorable conditions previously mentioned.

Of the varieties experimented with, *Culex pipiens* is without doubt the transmitter of this infection.

As bases for the conclusions arrived at in this paper, I wish to mention the following facts:

1. The female alone is capable of sucking blood.
2. The object of the blood is to assist in ovulation.
3. The insect does not perish after laying her eggs.
4. The duration of life of *Culex pipiens* is generally about two or three months.
5. The insect may bite every four or five days. It digests its blood meal in three or four days.
6. The female mosquito, kept in captivity, feeds after its first blood meal, on several kinds of food. Some experimenters feed them on fruit. In our laboratory we use sugar. A female mosquito will feed on sugar before she has digested the blood. I have dissected insects whose true stomach still contained blood and whose accessory stomach, or esophageal diverticulum, was full of syrup.



## HISTOLOGICAL TECHNIQUE.

For studying the distribution of the filariae in the body of the mosquito, sections are best used.

On the other hand, if we wish to follow the cycle of development of the parasite, of its dimensions and structure, we must dissect the infected mosquitoes.

## TECHNIQUE FOR THE PREPARATION OF SECTIONS.

1. The live mosquito is placed in absolute alcohol. In this manner the penetration necessary for fixation and dehydration is obtained. Duration 24 hours.
2. Removal of wings and legs in the same liquid, in a watch glass.
3. Equal parts of absolute alcohol and ether, 24 hours.
4. Weak solution of celloidin, 24 hours.
5. Thick celloidin, 24 hours.
6. Mounting on block.
7. Chloroform, 20 minutes.
8. Eighty per cent alcohol until ready to cut.

The sections were always stained in Böhmers hematoxylin, followed in some cases by eosin and in others by Van Gieson's solution.

## DISSECTION.

The living insect is placed in a test-tube with a small amount of water, and is shaken until the wings become wet, and the insect floats on the water. The liquid is poured into a watch glass, where the legs and wings are pulled off by means of forceps.

The further dissection should be made in the following order:

Dissection of the abdomen upon a slide, constantly irrigating the mosquito with distilled water or weak salt solution. After dissection place under microscope and determine by medium power whether there are any filariae. The head and thorax are transferred to another slide. Here the chitin is broken near the neck, and the head is transferred to another slide. Each part is thus dissected separately, and we are able to determine the localization of the parasites. If the latter be well advanced in their development, they push their way out through the openings made in the chitinous covering at the time of dissection.

If we wish to preserve the specimens, the filariae, if they are large enough, are transferred from place to place upon the slide, and repeatedly washed, while we clean and remove all detritus of the body of the mosquito from the slide. We finally add a drop of mixture of water, glycerin, and formalin, and the preparation is covered and cemented. Such preparations may be studied with the immersion lens.

When the filariae are too small to be isolated, it is best to leave them in the midst of the detritus, and to stain them as follows:

1. Allow them to dry on the slide.
2. Fix with 95 per cent alcohol, 1 to 2 minutes.
3. Place in water a few minutes.
4. Böhmer's hematoxylin, 1, 2, or 3 minutes, according to the intensity of the stain desired.
5. Wash in running water, 3 to 5 minutes.
6. Watery .01 per cent solution of eosin,  $\frac{1}{2}$  to 1 minute.

7. Wash in 95 per cent alcohol.
8. Dry with paper.
9. Oil of cloves, 2 to 3 minutes.
10. Xylol.
11. Balsam.

The above technique may appear complicated, but it may be carried through in 8 to 10 minutes. Without staining, the filariae, when they are too small or too few, may escape observation.

The dissection above described is not a careful dissection of the insect, but should be considered rather as a coarse fragmentation into sections, as follows: the stomach, intestine and ovaries in one piece; the thoracic muscle in four or more bundles; and the head and mouth parts. Care should be exercised not to lose a single fragment.

### METAMORPHOSIS OF FILARIA IN MOSQUITO.

I shall describe now the several steps in the metamorphosis of the filaria in the mosquito. I shall touch upon some points that have been in dispute, but upon which my opinions are free from all bias, since my observations were made before I had a complete knowledge of the literature.

#### CHANGES OF POSITION OF THE FILARIA WITHIN ITS HOST, AND THE DATES COUNTING FROM THE FIRST DAY OF INFECTION, THAT CORRESPOND TO SUCH CHANGES.

Histologically we may say that the filariae are found in one or the other of two structures, namely: in the stomach, together with the blood ingested from an infected patient; or in the loose connective tissue, called the fat body.

Topographically we find that filaria embryos may occupy the following positions: (1) they are taken into the stomach where they remain during a variable number of hours; (2) they then pass to the thorax where they lie as in a nest, undergoing the principal morphological changes; and (3) they usually pass out through the head and labium, but sometimes the worm loses its way in the body and becomes lodged in the abdomen under the chitinous covering.

The transformations of the filaria may be considered as follows in my several series:

*First and second series.*—At the end of 36 hours all the embryos had left the cavity of the stomach, and had wandered into the thorax. At the end of 12 days they were found in the neck.

*Third and fourth series.*—In 32 to 40 hours the embryos had passed to the thorax. As an example of unusual precocity I may mention one instance in which an embryo had found its way to the thorax 13 hours after the ingestion of blood in the stomach.

In these series filariae reached the neck 13 days and 15 hours, and the labium 15 days and 14 hours after infection (stained preparations similar to those of Low). On the 20th, 30th and 38th day filariae were still found in

the labium. One of the insects of this series presented, 15 days and nine hours after infection, 10 filariae in the head, and only three were still in the thorax.

*Fifth series.*—As in the other series nearly all the embryos had already left the stomach at the end of the first 40 hours. I should mention, however, one mosquito in whose thorax 21 filariae were found, and whose stomach filled with liquid blood still contained eight worms three days and nine hours after infection.

In this series the worms were still found in the thorax after 15 days and 14 hours, and their metamorphosis was not quite completed. They reached the head at the end of 19 days and nine hours. On the 30th and 38th day they were still visible under the microscope, moving in the labium of the living insect.

*Sixth series.*—In this series the filariae reached the head after a period of 22 days and eight hours.

To recapitulate: In the first four series the filariae reached the head in 15 to 16 days. In the fifth series some delay was noticed, since filariae were found in the stomach after three days and nine hours, and none were found in the head on the 17th day. In the sixth series the worms reached the head and labium on the 22d day.

This difference in the time of migration is due to the fact that the embryos do not migrate until they have completed certain phases of their cycle of development. We shall consider later on the cause of this delay of development in some cases.

#### DIMENSIONS AND GENERAL CONFIGURATION OF THE EMBRYOS IN THE DIFFERENT STAGES OF DEVELOPMENT.

The morphological changes were found to vary in the different series in the same manner as the migrations of the worm.

In the following table all the series are presented together, and the maximum time, that corresponds with the several changes, is given.

The dimensions of the embryo filaria in the human blood are: length, from 0.130 to 0.300 mm; width, 0.007 to 0.011mm.<sup>1</sup>

##### DIMENSIONS OF THE FILARIA EMBRYOS.

	Length	Width
In the stomach of the mosquitoes a few hours after ingestion - - -	0.203-0.277 mm.	0.0055-0.0074 mm.
In the body of the mosquitoes during the first three days - - - }	{ 0.240 mm. - 0.208 mm. - 0.203 mm. -	{ 0.0121 mm. 0.0111 mm. 0.0148 mm.
In the three following days up to the sixth day - - - }	{ 0.192 mm. - 0.176 mm. - 0.160 mm. -	{ 0.0185 mm. 0.0194 mm. 0.0185 mm.
In the three following days up to the tenth day - - - }	{ 0.264 mm. - 0.280 mm. - 0.336 mm. -	{ 0.037 mm. 0.0407 mm. 0.0333 mm.
During the following days the filaria continued to grow until it reached its full development on the 12th day in the more rapid, and the 22d in the slower series - - - }	{ 1.408 mm. - 1.440 mm. - 1.504 mm. -	{ 0.024 mm. 0.024 mm. 0.032 mm.

<sup>1</sup> Dr. M. Braun, *Die Thierischen Parasiten d. Menschen*, Würzburg, 1903, p. 265.

A careful study of the above figures will show that the development of the worm occurs in two stages. During the first stage the embryo grows shorter until it measures 0.160 mm., and then it grows longer, and attains the maximum of 1.504 mm. The width, on the other hand, changes in the opposite direction: during the first stage, it increases from 0.012 mm. to 0.018 mm. Finally, both dimensions increase; the length, much more rapidly than the width.

With these changes in size we have also morphological changes. The shape of the embryo in the human blood is slender and delicate. After the ecdysis in the stomach of the mosquito, and soon after the migration to the thorax, the embryos become plump and sausage-like. Towards the end of the cycle of development the worm grows gradually longer and, though much larger, assumes again its original shape.

#### STRUCTURAL CHANGES DURING THE CYCLE OF DEVELOPMENT.

Fig. 1, A, gives a good idea of the hyaline sheath, fitting closely around the body of the embryo, but extending considerably beyond its length.

The embryo presents a somewhat square rather than rounded head. The posterior extremity is quite pointed. About one-third or one-fourth the length of the worm from the anterior extremity there is a clear space, the V-shaped space described by Manson. There is much doubt as to the embryonic significance of this space. A similar one is also found towards the posterior extremity. These spaces are quite apparent in stained preparations. In fresh preparations they appear as highly refractive spots.

In living embryos there is a remarkable series of refractive points or granules extending, rosary-like, from the middle of the embryo to about one-quarter from the posterior extremity. The whole rosary moves backwards and forwards, but never advances beyond the center of the embryo.

The examination with high powers reveals a distinct transverse striation of the body (Fig. 2).

The anterior extremity or mouth has been described in divers manners. The highly refractive character of this region gives rise to much uncertainty. I have not been able to form any positive opinions as to the character of the structure. In one instance only was I convinced of the existence of an arrangement consisting of three prismatic teeth. These teeth had the appearance and color of the teeth found in the maxillae of mosquitoes and somewhat yellowish color, such as is found in chitinous structures. The observation, however, is too isolated to warrant any final conclusions. The mobility of the living worm renders the observation more difficult. In stained preparations we see simply a sheath filled with minute granules.

The posterior extremity becomes remarkably changed during the metamorphosis. From being sharp and pointed, it becomes the widest part of the worm.

Upon leaving the stomach of the mosquito, the filaria leaves its sheath in the coagulated blood. This operation is called ecdysis.

The first change visible after arrival in the thoracic muscles is a slight narrowing of the body of the embryo near its posterior fifth or sixth. This progresses slowly, and, at the same time, there is a gradual invagination of the



posterior sixth, into the anterior portion. The worm grows shorter and thicker (Fig. 3).

The invagination continues until there is only a small portion of the pointed extremity protruding, like a small appendix from the thickened and rounded extremity.

The anterior extremity is somewhat square-shaped. The mouth, at this time, constitutes a round opening leading into a funnel-shaped cavity. At the narrow termination of the latter we find the beginning of the digestive tract. This is now perfectly visible, extending to the anus. It becomes, however, more prominent when the filaria begins to elongate.

During this stage of elongation, the cephalic extremity does not change; excepting that, in some instances, a small, stiletto-like body is seen projecting from the oral funnel. The posterior or appendicular extremity suffers a retrograde metamorphosis. Although the worm has lost its outer sheath in the process of ecdysis, it is still surrounded by an adhering hyaline membrane. This fine integument, first described by James, becomes quite visible when it swells by imbibition. The structures contained within this membrane, at the end of the appendix, are seen to disappear, leaving an empty portion at that point. This portion appears then in the shape of a very small triangular, hyaline cap, upon the rounded posterior extremity.

The intestine can now be seen consisting of three distinct portions: one extending 0.384 mm. backward from the bottom of the oral funnel. Here we find a constriction which reminds us of the esophageal bulb. From this point the intestine continues, forming a slightly undulating line to the anus. The latter appears at varying distances from the tail end, as may be seen in the following table:

Length of Filaria	Width of Same	Distance from Anus to Tail End
0.192 mm.	0.0185 mm.	0.021-0.0240 mm.
0.336 mm.	0.0333 mm.	0.0259 mm.
0.640 mm.	0.0370 mm.	0.0444 mm.
1.344 mm.	0.0320 mm.	0.056-0.0690 mm.

Apparently the distance of the anus from the cauda increases; but in relation to the increase in length of the worm the distance really diminishes.

The diameter of the anal opening is greatest when the filaria is shortest; it becomes narrower and filliform as the embryo reaches its complete development in the mosquito.

I have seen a peculiar viscid substance leaving the anus, and remaining adherent to the filaria for some time. The substance appears at times to be inclosed in transparent vesicles.

As the embryo reaches its maximum length, the final changes occur in the caudal end; namely, the development of the three lobes. These are at first very small, but become later quite prominent. Sometimes they give the impression of a hook formation; but they are rounded, and symmetrically arranged around the point.

I desire to call attention to a peculiar structure observed by me in three of the embryos examined. It was observed in the stage just preceding the final development. I do not mention it as a regular stage in the growth of the worm because I have only observed it in a few instances.

This peculiar structure presents itself at the caudal extremity. It appears in the shape of a tube which starting from the posterior end, extends forwards, bending somewhat, and protruding sidewise at a short distance from the anus, where it projects forming a small conical protuberance (Fig. 4). The whole tube can be seen through the transparent tissues, and presents the shape of a sleeve, the cuff of which forms the conical elevation. This peculiar structure is quite motile. By movements that may be described as erectile the sleeve-like organ is seen to bend, causing a lateral swelling of the terminal portion of the body of the filaria. At the same time the small titlike protuberance moves nearer or further from the anus. In this protuberance we are able to observe that the organ in question is hollow.

In a filaria measuring 1.344 mm. in length, and 0.032 mm. in width the following measurements were made (Fig. 4):

Distance from the anus (a) to the caudal extremity (b).....	0.069 mm.
Distance from the anus (a) to the nearest edge of the protruding cone (c).....	0.009 mm.
Distance from the tail end (b) to the nearest edge of the protruding cone (c).....	0.045 mm.
Width of the protruding cone at its base.....	0.015 mm.

The titlike protuberance measures 0.0105 mm. in height on the caudal side, and 0.006 mm. on the side toward the anus.

During the erection the distension of the posterior portion always occurs on the side opposite the titlike projection. This expansion may extend to a distance from the tail end varying from 0.0525 mm. to 0.069 mm.

In measuring from the tail end the rounded extremity, not the appendix, was taken.

These measurements were made on living filariae anesthetized by adding a few drops of ether to the water.

I am not able to give any functional significance to this structure. I am sure that it is independent of the anus. Though no spicules are found in connection with the organ, the idea suggests itself that it may have some relation to the sexual organs.

The description of the steps in the metamorphosis of the embryo in the *Culex pipiens* may be condensed as follows:

1. The insect sucks the blood of a patient infected with filaria.
2. The embryo loses its sheath in the stomach of the mosquito, ecdysis.
3. Migration from the stomach to the thorax. This migration always takes place through the gastric wall, since both orifices of the gastric dilatation are completely closed when the stomach is full. The embryo leaves its sheath in the gastric contents or caught in the wall of the stomach, where it is left at the moment of exit.

4. The embryo rests in the thorax, and goes through the following transformations:

- (a) Narrowing and invagination of the tail.
- (b) Invagination continues and the embryo grows shorter and wider.
- (c) Widening and shortening continue, and the invaginated portion forms a hyaline appendix.
- (d) Period of growth and formation of the three lobes.

The motility and non-motility of the embryo constitute biological features that are characteristic for the various phases of its development.

The active motility of the worm in the human circulation is further increased when the worm reaches the stomach of the mosquito. Upon reaching the thorax, however, all active motion ceases, and we only observe now and then very slight and sudden lateral movements. When the worm is nearing the maximum of its growth we notice a slight increase in the lateral movements of the anterior extremity. As soon, however, as the embryo has completed its metamorphosis in the body of the mosquito, it recovers its motility, in order to accomplish its migration to the head. There the filaria awaits the opportunity to complete its cycle of development in the human host.

This latter motility is peculiar in that it enables the worm to push its way in the midst of soft tissues. It is a kind of motion that is quite distinct from that of the earlier stages when the animal has to move in liquids. I shall dwell further on upon these differences and shall use them in support of my views.

The worm, then, shows its activity in order to enter and to leave the thorax, and while in the latter it lies quiescent during its metamorphosis.

It happens sometimes, though rarely, that when the filaria reaches its maximum size, and starts on its way to the head, it may mistake the route, and wander towards the caudal extremity. The worm, however, will always keep in the fatty tissue, and close to the chitinous covering. These stray worms all proceed from the thorax. I have never met with a single embryo undergoing the process of metamorphosis in any other structure than the thoracic muscles.

From three principal characters we may conclude that the filaria has completed its cycle of development in the mosquito, namely:

1. The arrival in the labium.
2. Complete development of the three lobes in the caudal extremity.
3. Active motility.

I desire now to consider the marked differences that were noticed, with respect to the time consumed in the cycle of development in my several series.

In his earlier investigations Manson saw embryos already in active motion seven days after infection. Bancroft<sup>1</sup> says he never saw them before the 16th or 17th day, and in cold weather, even the 20th; James<sup>2</sup> asserts that from 12 to 14 days are required for the complete metamorphosis, but he admits that, in localities where filaria abounds there may be species of mosquitoes that are peculiarly favorable to the development of the embryo. In this way he explains the seven days mentioned by Manson in his earlier work. He mentions the influence that climate and other factors may have upon these changes, and he reminds us of the fact that some of Manson's mosquitoes were kept in the incubator at temperatures ranging between 27° C. and 29.5° C.

We should not forget that the different results above mentioned were obtained in different countries. On the other hand I have also obtained the same variability in the results, though I was working in one locality, in the same laboratory, and with the same kind of mosquitoes.

The different results obtained by me, experimenting always with the same kind of mosquitoes, show that we cannot accept the suggestion of James to the effect that there may be species of mosquitoes that favor the development of the filaria.

Different species of mosquitoes may serve or not as hosts, but if the insect is capable of acting as such, the time limit of the cycle extends within bounds that have not yet been fixed.

In my series of experiments the only variable point was the season of the year. The results obtained were the same for each

<sup>1</sup> THOS. L. BANCROFT, *Jour. of Trop. Med.*, 1899, 2, p. 96.

<sup>2</sup> S. P. JAMES, *Jour. of Trop. Med.*, 1900, 3, p. 46.



series and the variations were noticeable only in the comparison of one series with another. The variations correspond, therefore, with the changes of temperature that prevailed at the time of experimenting with each series.

From the official bulletin of the meteorological station I have been able to obtain the mean temperature prevailing during the time of each series of observations, and I present the results in the following table.

MOSQUITOES (*Culex pipiens*).

Series	Dates	Complete development of the filaria in	Mean temperature for each period
1, 2, 3.....	August 25 to Sept. 28 }	15 days	25.5° C.
4.....	October 12 to Oct. 27 }		
5.....	Nov. 9 to Nov. 26 }	19 to 23 days	22.5° C. }
6.....	December 13 to Jan. 4 }		
			21.2° C. } — 21.8° C.

It is evident from the above table that the temperature is the chief factor in modifying the cycle of development. Heat is, then, the climatic condition that favors the development of the embryo.

The daily oscillations of the temperature were nearly the same during the time of the several series. The daily range was of 4.4°, 4.5° and 4.1°. The daily oscillations in Cuba, therefore, do not influence the growth of the worm.

I am preparing a series of experiments with mosquitoes kept in incubators at a uniform temperature, in order to fix the extremes of most favorable, and absolutely unfavorable temperatures.

#### THE ESCAPE OF THE WORM FROM THE INTERMEDIARY HOSTS.

The several kinds of living beings that may act as transmitters of disease, may be divided into two great groups. In one the infecting agent must go through a cycle of development within the host, without which the further transmission to another animal is impossible. In the other group the parasite, without undergoing any developmental changes is mechanically carried from one animal to another by an intermediary animal acting as an indifferent vehicle.

However, the act of infection by the first group of hosts may occur either actively or passively. In the first instance the con-

veying animal, by its own act, inoculates the infecting agent; in the second, the intermediary host remains passive until the animal to be infected, takes up the infecting agent. An illustration of the first, or active mode of transmission, we have in the mosquito inoculating filaria by its own bite. We have in the hog, in whose flesh the cysticercus must wait passively until it is swallowed by man, an illustration of the second or passive mode of conveyance.

The filaria, leaving the body of man in the embryonal stage of its development must suffer a series of changes that can only take place in the body of the mosquito, before the worm can reach maturity, adult and sexual life, once more in the body of man; the mosquito (in my experiments *Culex pipiens*), is therefore the only transmitting agent.

When we take up the problem as to how this transmission is carried out, we find differences of opinion: some holding that the process is a passive one, and others that it is active. In the first instance it is maintained that the mature embryo passes from the body of the mosquito into drinking water, and is finally swallowed by man; in the second instance it is maintained that the mature embryo is directly inoculated by the mosquito. The former is the the old theory of Manson; the latter, the more modern of Low and James.

In attempting the solution of these problems, we must start with a thorough understanding of the facts presented in the earlier parts of this paper, specially with respect to the evidences of the final development of the embryo, its arrival in the labium, and the time that has passed since the mosquito used in the investigation became infected.

With all this information, I proceeded first to determine whether the filaria leaves spontaneously the body of the mosquito when the latter, still living, drops into the water, and care is taken that the integrity of the structure of the insect is preserved.

With this object in view I selected mosquitoes whose date of infection was well known, and in which the stage of development of the filariae was well established by the examination of other insects of the same series.

The selected mosquitoes were placed in water in a watch-glass, and studied under the microscope. The results were always negative. The mosquitoes died without discharging a single worm. Upon dissection of these insects at the end of 24 or 28 hours, all the filariae within them were found dead, which is also the case when they are allowed to remain a few hours (up to 18) free in water.

That the integument of the insect was preserved in these cases was shown by the exit of large numbers of infusoria as soon as the chitinous covering was broken in the act of dissection.

This experience proves that, **when a mosquito falls into the water if its cuticle be preserved, the filariae it may contain are unable to escape and perish by imbibition of water within a period of 24 hours.**

My second series of experiments was intended to determine whether the filaria escapes at the time when the insect is feeding, or drinking, or when it lays its eggs. Be it understood that in these acts we exclude especially the sucking of blood.

Six mosquitoes, from among those most seriously infected, were placed in a separate jar on the sixth day of their infection. The water in the jar was examined every day.\* The lumps of sugar hung up in little bags to feed the mosquitoes were also examined daily by dissolving them. The results of these experiments were always negative even up to the 38th day. At the end of this time while no filariae were found in the water, they were still to be found, living, and active, in the heads of the mosquitoes.

In other mosquitoes of the same series dissected at stated intervals, we were able to see that the filariae were fully developed, and had arrived in the labium on the 17th day.

My second experiment proves, **that the filaria does not pass from the living mosquito in the act of suction of water or sugar.**

Whether the worm will pass into other kinds of food such as bananas, as has been suggested in connection with *Filaria immitis* by Grassi and Noé, is very doubtful. If the worm could pass into bananas and dates, it surely would do so in sugar.

\*The daily examination of the water was made necessary in order to eliminate other organisms resembling the filariae that are likely to develop in water that has been standing some days.

This same series of experiments, and other observations, show that the filaria does not pass out at the time of laying the eggs.

There is one opportunity—the only one—for the filaria to find its way out of the mosquito into drinking water, and that is by accidental break in the integument; as may be shown experimentally in the act of dissection. But this must happen very rarely. Furthermore when the worms are set free in water they sink to the bottom and die by imbibition.

It appears then that only a series of accidents could bring the living filaria to the human stomach in the manner described.

The other hypothesis suggested to account for the transmission of the worm is that of a direct inoculation. There has been thus far no actual demonstration of the process.

Maitland argued that, "if it be true that the worm is introduced into the human body from the proboscis, we must suppose that the embryo must be always in position, ready to improve the opportunity given by the short time devoted to the act of suction." This is precisely what happens. The filaria is always ready.

The proof that the filaria remains day after day in the labium of the infected mosquito kept in captivity without a chance to repeat its feeding on blood, is found in the results obtained in sections by Low, and in the dissections made by James. These results suggested that the probable opportunity for escape is afforded in the act of drawing blood. The experiments here reported show that the migration does not occur during other acts of suction.

The experiments about to be described show how we may bring about at will the migration of the filaria, and permit the definite observation of the various steps in the process.

A mosquito is selected in which the filariae have reached their final stage of development, and are lodged in the labium. It is best to select an insect 28 to 30 days, or more, after infection. After removal of the wings and legs the insect is placed alive upon a slide and irrigated with a very weak salt solution. No cover-glass is employed. Under the microscope it is possible to see, through the transparent walls of the labium, the actively moving worms within that structure.



If no pressure\* is used, these movements may be watched for hours without observing the escape of a single worm.

Usually all the setae are enclosed within the labium, thus preventing a clear view of the movements of the filaria. In order to obviate this difficulty I press very lightly with the side of a needle upon the base of the proboscis, and then with the point of a needle inserted between the setae and the labium, I pry them further apart. The filariae can now be distinctly seen actively moving and agitating the two tracheal tubes. The worms occupy usually the proximal third of the labium; sometimes they extend further out.

The slide is now placed near the flame of a Bunsen burner. This must be done very carefully in order to avoid fatal overheating to the insect and the parasite. Watching the preparation, an unusual activity in the movements of the filariae is now to be seen, and the cephalic end particularly becomes agitated as if seeking a point of exit. This cannot be found laterally because of the chitinous covering, and the filaria advances toward the anterior extremity of the labium. If the liquid is allowed to cool, the movements become slower and even cease altogether. Upon warming carefully again, and adding more tepid fluid, the movements are revived until the worms reach the point of the labium. If now the application of heat is stopped, we find that the worms appear to feel around with their cephalic extremity, but fail to break out.

I am sure that there is no natural orifice at the terminal end of the labium, because if there were, the filaria, having reached this point, would find no obstacle to its exit. And, furthermore, a careful study of the extremity fails to discover any orifice.

I cannot agree with Grassi and Noé, quoted by Dutton, who

\*Bancroft, in experimenting with *Filaria immitis*, cut off and mounted on a slide under a cover-glass the proboscis of an infected mosquito. By making slight pressure upon the cover-glass he forced the setae out from the labium. He observed under the microscope that upon increasing the pressure the actively moving worm protruded from the extremity of the labium. "Whether there be at this point," says Bancroft, "a natural opening, appears to be doubtful; but every time that this experiment was tried the worm made its exit from the point and never elsewhere."

However, when we compress the labium, a rupture of its anterior extremity occurs, and the exit of a considerable amount of the fatty contents takes place. The break occurs at the point mentioned because of the thickness and unyielding character of the tissues at the end of the labellae. It is the break which offers a place of exit for the worms.

suggest that "when the mosquito, in biting, pierces the skin, there is a rupture at the point, through strain, and the filaria escapes. They imply that the crowding of the filariae causes the strain and distension of the tissues.

In my experiment there is no skin to pierce. Furthermore, the previous extrusion of the setae has really diminished the strain at this point. Nor can there be any tension when we have only one or two filariae with plenty of room. Besides, we do not have the bend or elbow formed by the labium in the act of biting, and which might contribute to the strain suggested by Grassi and Noé, for the labium lies perfectly straight. Even when the number of the filariae is great there is no irregular rupture anywhere in the labium, as would result from over tension.

If the heat is kept up carefully and steadily we can see the embryo seeking the point, making pressure there, and finally perforating the cuticle at a certain point.

This perforation is made quite suddenly, for we see the cephalic end jump out, as it were; the rest of the worm following slowly by serpentine movements. As soon as it reaches the fluid the serpentine movements continue, but the worm ceases to be able to advance. This remarkable difference in the results obtained by the movement indicates that the soft tissues at this stage are the natural element of the worm, and that it is prepared to move on into the human tissues directly from the mosquito. In water, on the other hand, the filaria not only cannot live, but cannot even move from place to place.

In their exit the worms follow the regular order in which they occur in the labium and head. We have seen two worms making their exit at the same time (Fig. 6).

Occasionally, after two embryos have been started out, it has been necessary to warm the liquid again in order to bring other worms down from the head to the labium. Of course, after the first embryo has broken out, the others find their way out with greater readiness.

What temperature is best adapted to stimulate the passing out of the filaria, I am as yet unable to say, but I am inclined to think that the temperature should be rather high. A slight vapor

should rise from the liquid, just enough to dim for a moment the lenses of the microscope.

Naturally enough the thought at once suggests itself of the importance of the heat in human blood as a factor in determining the exit of the filaria at the moment of biting.

#### POINT OF EXIT OF FILARIA.

Dutton describes a triangular space (seen in sections) near the point of the labium, and limited above by the chitinous band that forms the upper surface of the labium, and upon which the setae rest. He then adds: "This region appears to be the weak point in the chitinous exoskeleton of the labium; and it is probably at this point that the filaria escapes during the act of biting."

A careful study of the anatomy of the anterior extremity of the labium gives the following results:

The extremity of the labium presents an articulation indicated by a transverse groove (Fig. 5). The general appearance of this articulated end reminds us of the cloven hoof of certain animals. It is also divided symmetrically by a longitudinal groove, into two parts, called labellae. The separation, however, affects only the distal half of the end piece. In the proximal half the two sections are united by a membrane which, similar to an interdigital web, allows of free motion.

These two lateral bodies or labellae are cushioned with fatty tissue. The external (lateral) covering is chitinous and is rather thick. The internal (median) lining is a soft membrane covered with hairs.

I should mention that the whole inner lining of the labium is non-chitinous. It is a soft membrane made up of rounded cells. The filariae are lodged between this membrane and the outer cuticle.

When both labellae are united the longitudinal groove disappears and the whole structure appears as one piece.

Each labella has its own independent motion. When both segments are separated as far as the membrane (Fig. 5, B) will permit, we observe another complicated structure which protrudes pointlike between them, and forms with them a sort of tripod

(Fig. 5, a). This central portion appears to be a sort of buttress or rib in the interlabellar membrane. It consists of a strong chitinous groove (Fig. 5, b) along the median line, and two (c) lateral, hairy portions, of semicircular shape, partially covering the groove. Between the two lateral portions there is seen an obtuse point, which represents the protruding posterior wall. The whole structure, excepting the groove, is formed of a delicate hairy tissue.

It is difficult to understand the object of this median portion. The hairy covering suggests a tactile function; but it is also possible that the object of these structures may be to bring about a close approximation of the epi- and hypopharynx.

All these well cushioned structures of the labellae are evidently intended to hold together and control the other portions of the proboscis when they are being introduced into the skin, in the act of biting. The control thus exercised, permits a certain amount of sliding motion at the same time that the setae are held rigidly together. The terminal portion also covers up completely the spot where the lancets penetrate.

When we watch with a strong lens the act of suction, we observe at once the backward bend of the labium with the penetrating setae forming the chord of the arch. The bend or elbow formed by the labium becomes the more acute, the greater the depth to which the penetrating elements are introduced. If the depth be small, at least one-third (terminal) of the length of the labium remains closed, forming a cylinder; but if the insect is obliged to push deeply in search of blood then the whole labium as far as the transverse furrow enters into the formation of the arch, and the penetrating elements of the proboscis are held together solely by the labellae which bend downward and backward at the articulation, and are placed in a position parallel with the penetrating setae.

At all events the extreme end of the labium is applied in very close contact with the skin surface at the point of penetration of the setae.

The filaria makes its exit always from one of the lateral points, that is, from the tip of one or the other labella. At these points,



on the median aspect, the hairy integument is quite delicate, and readily perforated by the worm.

The place of perforation is, therefore, in close contact with the penetrating wound of the skin, and the setae. The latter are in constant motion (even movements of dilatation), and the best opportunity is offered for the successful inoculation of the parasite.

Having noted the extraordinary rapidity with which the filaria makes its exit, in one case five worms were seen to push their way out in less than one minute, it seemed important to determine the duration of the act of blood sucking. The minimum of time was found to be a minute and a half, and the maximum four minutes. The mean time was two minutes; or more than enough for the mosquito to discharge its whole load of filariae into the wound.

Fully understanding all these phenomena, we cannot help but be impressed with the facility with which filarial infection is brought about, when the conditions are favorable.\*

Fortunately the non-experimental infections frequently miscarry through failure of perhaps the most insignificant detail in the evolution of the parasite.

#### EXPLANATION OF PLATES.

(Original drawings by the author.)

##### PLATE 1.

FIG. 1.—A. Embryo of *filaria sanguinis hominis nocturna* in human blood.

B. Sheath of the embryo in the same preparation.

FIG. 2.—The same embryo of Fig. 1 under the immersion lens. The transverse striation of the cuticle is distinctly seen.

##### PLATE 2.

FIG. 3.—1. Embryo in human blood—2 to 7. Stages of development with measurements of the embryo in the mosquito. The relative sizes represented in the figure are exact, and represent amplifications of 70 diameters. (a), (b), (c), Process of invagination in the tail end of the embryo, seen as the worm passes from the sharp (1st) to the appendicular (2d) form.

FIG. 3'.—Anterior (a) and posterior (b) extremities of a fully developed embryo in the mosquito; (c) anus.

\*I have gathered mosquitoes from the room occupied by the patient, the subject of my experiments, and I have found filariae in the process of evolution. It would be interesting to examine systematically the blood of those individuals who have lived in contact with the patient.

FIG. 4.—Peculiar formation seen in the caudal extremity of some embryos at the termination of the mosquito period of development; (*a*) anus; (*b*) posterior extremity where the three characteristic lobes present themselves; (*c*) projecting tip forming the end of the sleeve, (*c*); (*d*) portion of the sleeve that expands with the erectile movements of the same.

PLATE 3.

FIG. 5.—A and B. Extremity of the labium. Anatomic study to show the point where the filariae make their exit. In Fig. A the lateral portions (labellae) are in contact; but in Fig. B they are separated to show the special structure (*a*).

C. Special structure composed of (*a*), extreme blunt end; (*b*) chitinous groove; (*c*) lateral surfaces covered with fine hair.

FIG. 6.—Reproduction from nature of the exit of the filaria at the place of selection at the end of the labium.

PLATE 1.



FIG. 1.



FIG. 2.





PLATE 2.

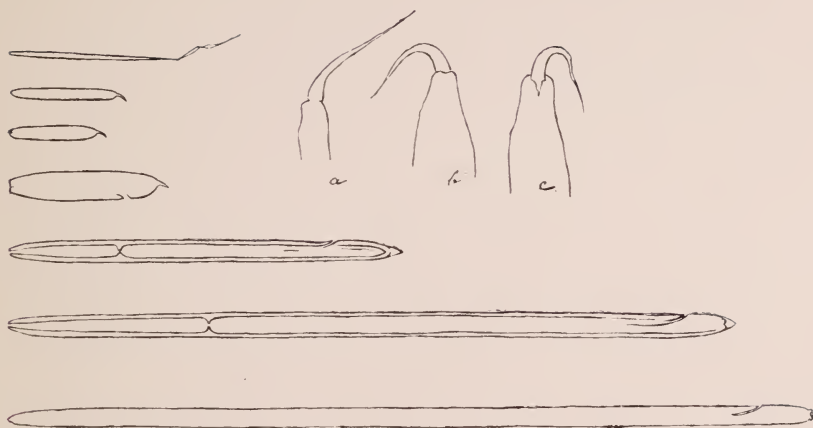


FIG. 3.

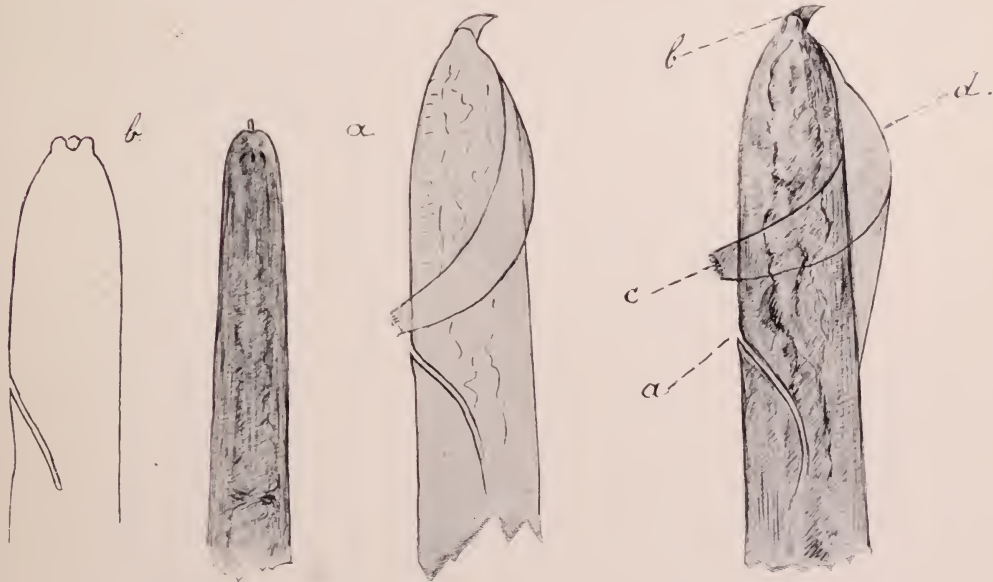


FIG. 3½.

FIG. 4.



PLATE 3.

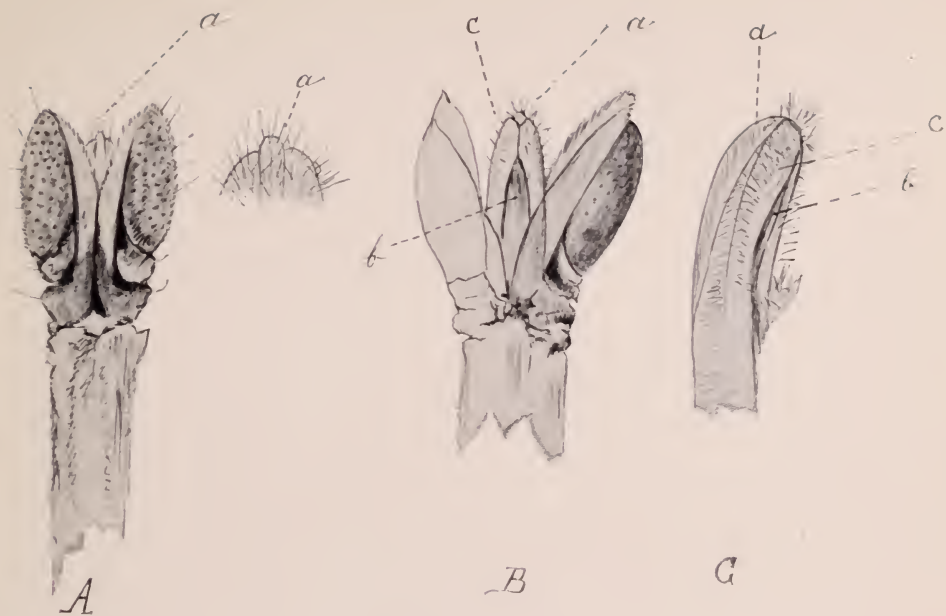


FIG. 5.

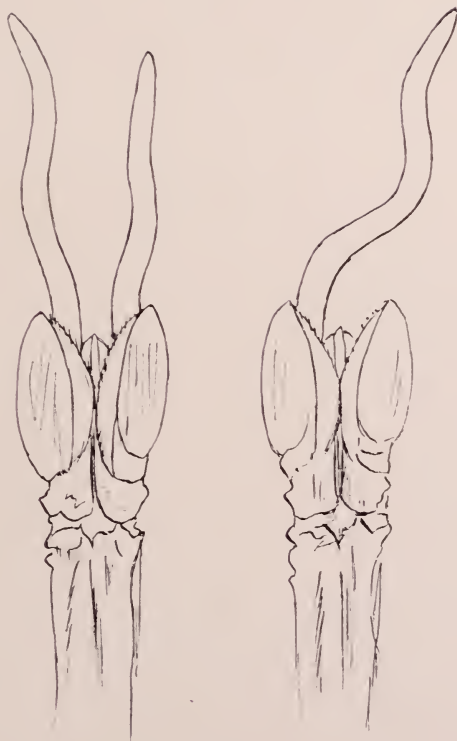


FIG. 6.





## A POSSIBLE CAUSE OF THE FORMATION OF GAS IN CANS OF CONDENSED MILK.

CHARLES WRIGHT DODGE.

In the preparation of condensed milk it is sometimes found at the end of several weeks after the milk has been sealed up in the small tin cans that the ends of the latter bulge because of the formation of gas within the cans. Samples in this condition were received for examination with directions to pursue the investigation along bacteriological lines since experiments made by a competent person had shown the cause not to be chemical action.

The results which are summarized in this paper are derived from a study of the cause of this formation of gas.

When normal and bulged cans with their contents were compared it was found that while the interior of the former was bright and the milk had a normal taste and odor and gave only a faintly acid reaction with litmus, the inside of the latter was discolored and darkened, the contents having a cheese-like taste and odor and a strongly acid reaction. A preliminary test was made by inoculating fermentation tubes of neutral dextrose broth with milk taken from a badly bulged can, but no fermentation occurred. A test with milk from a sound can gave a similar result. On the supposition that the gas-forming organisms had perished in the former and were absent from the latter can, milk was used from one that showed only a slight amount of gas formation and from this four species of bacteria were isolated. These were tested singly and in combination with each other under a variety of circumstances and in numerous media, including diluted condensed milk, ordinary milk, lactose broth, dextrose broth, saccharose broth, and in combinations of these in different proportions with each other; in acid, neutral and alkaline media; at the room temperature and in the incubator; in the light and in darkness; in atmospheric air, in hydrogen and in carbon dioxide. Under none of these conditions did fermentation occur. Failing to find the offending organism among the four isolated, tests were

next made as mentioned above using the milk itself directly from a slightly bulged can, but no gas-formation took place. All tests were then repeated several times and were allowed to stand from two weeks to several months, but the results were always negative, and the conclusion was reached that bacteria are not the cause of the evolution of the gas.

By this time the supply of spoiled milk had become exhausted and recourse was had to butyric and lactic acids. It was learned that when dilute solutions of these in strengths varying from 1 in 200 to 1 in 500 in distilled water are allowed to act upon the metal of which the cans are made a slow evolution of gas takes place, its rapidity being inversely as the dilution of the acid. One-half c.c. of gas was formed at the end of two weeks in a tube of 15 c.c. capacity when a solution of 1 in 200 of lactic acid was allowed to work upon a piece of the tin having the diameter of a five cent piece, the tin becoming discolored, of course, during the process. In greater dilutions the production of gas is, naturally, slower in rate and less in quantity. If the same action takes place in the cans of milk the bulging is easily accounted for, but the exhaustion of the supply of material renders conclusive proof impossible at this time. It is probable that in the instance cited the gas was formed not by the bacteria directly but by electrolytic action between the metals of which the cans were composed and the acids generated by the growth of bacteria in the milk before the latter was "condensed."

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of the American Public Health Association  
at the Boston Meeting,  
Sept. 25, 1905*

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# *The* Journal of Infectious Diseases

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*Supplement No. 2, February, 1906.*

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## SOME OBSERVATIONS UPON THE AGGLUTINATION OF BACTERIA.\*

WILLIAM HALLOCK PARK.

My purpose in this address is to give briefly some observations upon the value of the agglutination test in establishing the identity or relationship of bacteria and in detecting the variety of bacteria exciting disease in cases of bacterial infection.

During the past three years, in connection with Dr. Katharine R. Collins, I have been more or less occupied in the study of these questions, and in this time I have learned much concerning the difficulties of properly interpreting the results, and of the limitations to the value of the agglutination reaction. It is my hope that a review of some of these experiences may be of interest. Before taking up the discussion of the two topics I wish to touch on some of the points to be thought of in the technique of carrying on the tests.

### SOME IMPORTANT POINTS TO CONSIDER IN MAKING AN AGGLUTINATION TEST.

1. The quantitative nature of the union between bacteria and agglutinin. This necessitates that with increase in the number of bacteria in the serum dilution there is more material to combine with the agglutinin. A thick emulsion of bacteria is therefore not agglutinated in as high dilutions as a thin emulsion.

2. The varying sensitiveness of the same variety of bacteria from day to day, even when grown from the same stock culture. At

\*Address of Chairman of the Laboratory Section American Public Health Association.

times no explanation can be given for this variation, but of its occurrence there can be no doubt.

3. The increased rapidity of union of bacterial substance with agglutinin as the temperature rises from  $0^{\circ}$  to  $37^{\circ}$  C. This necessitates that not only the time at which readings are made should be stated, but also whether the reaction took place at the temperature of the ice box, the room, or the incubator.

4. The greater height of the reaction, when long rather than very short periods of time are allowed for its development, provided the test is so carried out that the bacteria do not multiply in the agglutinating fluid.

5. The absence of reaction at times in low dilutions, with presence of reaction in higher dilutions. This phenomenon appears to be due to substances in the serum other than agglutinins. It rarely occurs in dilutions of serum above 1:50.

6. The growth of some varieties of bacteria in the serum-dilutions when the temperature allows of it, thus altering the proportion between bacterial substance and agglutinin. A good reaction may thus disappear in the course of a few hours.

7. The individual judgment in the estimation of what constitutes a certain degree of reaction. No two observers read the completeness of a reaction exactly alike.

8. The alteration in the test serum when it is used over considerable periods of time. A gradual deterioration takes place in the agglutinin in the serum. This is more rapid in diluted serum and with increase of temperature.

9. The medium in which the bacterial suspension is made, whether broth or salt solution, whether it contains sugars or not, etc., is of importance. Growth in glucose media, for instance, makes bacteria more sensitive and tends to natural agglutination. Broth as a medium for diluting serum gives usually a quicker agglutination than does salt solution.

10. The effect of heat and of some preservatives, when they are used, in altering the serum both quantitatively and qualitatively.

11. The considerable difference in the readings made macroscopically and microscopically. A most striking example of the difference in reading is seen in the method used by Dunham<sup>1</sup> and

<sup>1</sup> *Jour. Infect. Dis.*, 1906, Supplement No. 2. p. 10.



that by us. With meningococci examined microscopically after three hours in the incubator we obtained readings of a reaction of 1:200, while Dunham, using light suspensions in the ice chest for 36 hours, obtained a reading of 1:2,000. Both methods were probably equally correct, and the readings by one method could be compared with each other but not with those of the other method.

12. The observation, during the time of experiment, of the control specimen, and of any tendency to natural agglutination.

13. In absorption tests, when filtration methods are employed, the obstruction to the passage of agglutinins of the filter and of the bacteria coating the filter must be fully allowed for, as the coating formed by the various varieties of bacteria differs greatly in permeability.

#### AGGLUTINATION CHARACTERISTICS AS A GUIDE TO THE CLASSIFICATION AND IDENTIFICATION OF BACTERIA.

It has been unmistakably demonstrated that an agglutinating serum is composed of a number of agglutinins which owe their origin in the animal to the stimulus of the different proteid substances contained in a single cell or in several varieties of cells.

We have many facts which serve to point out the value of partially similar agglutination among bacteria in suggesting relationship such as between certain members of the typhoid-colon group of bacilli. It is true, however, that others which appear just as nearly related do not react to common agglutinins, and some that appear utterly unlike do react.

Thus Durham injected two animals with a different paratyphoid organism. These were obtained from two cases simulating typhoid fever and had the same biochemical activities. He found sera obtained from the two rabbits to have almost no similarity in agglutination. A serum clumping one 1:20,000 did not affect the other in dilutions of 1:100. The marked dissimilarity in the agglutinating characteristics of the bacilli contained in the colon group is another example. Among 14 strains of culturally characteristic colon bacilli isolated by us from 10 persons there were five distinct varieties, if classification were to be made by the agglutinating characteristics. In our recent investigations of pneumococci we have obtained a number of cultures from the exudate of characteristic

cases of lobar pneumonia, which have been alike in morphology, in action on inulin, on sugars, and in cultural characteristics, and yet they have differed absolutely in their affinity for agglutinins. This difference remains unaltered in the cultures as they are continued on culture media, and undoubtedly indicates a different chemical composition; but this is too intangible to be a sufficient reason for separating bacteria which appear to be alike in more essential points. At times, however, it may be very instructive. Thus in Dr. Goodwin's paper on p. 21 it is noted that from the nasal secretion of two healthy students a diplococcus was isolated, which, except in its agglutination, appears to be identical with typical meningococci derived from spinal fluid. This difference in the diplococcus excludes it from the type obtained from the epidemic cases, and even places it under suspicion as to whether it is a meningococcus at all. The complete identity in agglutination characteristics between organisms obtained from the nasal cavity of the sick and from the spinal fluid is strong proof of the former being not only meningococci but the same identical variety as that in the cord.

A species of pathogenic bacteria which develops only in disease is apt to give rise to later generations, all of which will be alike in their agglutinating characteristics, while one which has for the most part a saprophytic life is apt to give rise to distinct varieties. Under semisaprophytic existence the new generations are subjected to variable conditions, and thus become modified, so that, as in the case of the pneumococci, the streptococci, and the colon group of bacilli, we may have a continuance of the more striking cultural characteristics with such variation in the agglutinating affinities as apparently to call for separation into numerous varieties. This separation is useless, so far as we can now see, for any practical purpose, and impossible to define, as there would be no way for future investigators to compare their results unless the original culture or the specific serum was at hand.

The stimulation in an animal of agglutinins for any micro-organism is produced, according to our present views, by similar protoplasm in the infecting organisms. Some recent observations,

if correct, indicate that cells apparently widely separated have more or less common substances. Ballner states that a rabbit immunized with a pink yeast developed agglutinins for both typhoid and dysentery bacilli, so that they were agglutinated in dilutions of 1:1,000. A rabbit, immunized by us with yeasts, developed a serum which agglutinated paradysentery bacilli but neither typhoid bacilli or true dysentery bacilli. A less striking experience of our own was that of the serum of a horse, which after immunization with a paradysentery bacillus agglutinated both that bacillus and a typical colon in dilutions of 1:10,000. During injections specific agglutinins are first chiefly developed, but later the total amount of group agglutinins increases so as, at times, to equal the specific ones. The proportional amounts of group agglutinins for allied bacteria differed greatly at different times during the immunization of an animal and at the same time in different animals. Many conflicting statements are due to the lack of appreciation of this variability.

As the quantitative agglutination test usually fails to distinguish whether the reaction is due to specific or group agglutinins, use has been made of an absorption method to determine the action of the specific agglutinin which is present among the multiple agglutinins in the serum of every immunized animal. It has been fairly established that any bacterial strain which can absorb from a serum all the agglutinins which acted upon a certain microorganism, and which were stimulated by that microorganism, must be identical with, or extremely closely allied to, it. The virulence of the two microorganisms may, however, vary widely.

The technique of making the absorption test is rather difficult. When the agglutinating strength of the serum is high, large amounts of bacteria must be added again and again, or the serum must be highly diluted. In the latter case it is impossible to demonstrate that the absorption is complete. Usually the serum is diluted with four times its quantity of salt solution, and then mixed with about its weight of culture. After standing a few hours the mixture is centrifuged. If the supernatant fluid still contains agglutinins, more culture is added and the mixture treated as before. If the agglutinating strength of the serum has not been lowered below that of the

first absorption by the second addition of culture, it is certain that no further absorption by that culture is possible. If a culture is used which is identical with that used in immunization, all agglutinins will be absorbed if sufficient bacteria are added.

When the agglutinating strength is high the organisms may be removed by passage of the fluid through a Berkefeld filter. Here it must be remembered that the filter holds back most of the agglutinin until a number of c.c. have passed, and under certain conditions, as shown by Dunham, the close packing of the bacteria against the filter may continue to hold back agglutinin.

As a general rule it can be said that the agglutinins produced in an animal through the injection of any one variety of bacteria can be exhausted from the serum only by saturating it with sufficient quantities of that variety. In our experience not only the specific, but usually the common, agglutinins stimulated by it will thus be absorbed. All other varieties of bacteria will simply absorb any of the common agglutinins for which they have an affinity. If a serum is freed of all common agglutinins, it will clump only the variety of bacteria which was injected in the animal. It is practically impossible to remove absolutely all group agglutinins, since we only know that those having an affinity for the bacteria added have been removed.

The observations of Posselt and v. Sagasser,<sup>1</sup> that by the injections of a pure culture of one variety, agglutinins may be stimulated in large amount for other bacteria, which, however, cannot be absorbed by the variety used in immunization, have not been duplicated by us. The agglutinins not absorbed have been those present in the animal before immunization was begun, or those stimulated by the absorption of other substances. These agglutinins are abundant in horses and goats, especially for the typhoid-colon group. An agglutination of dysentery, paradysentery, and colon bacilli in dilutions of 1:1,000 has been met with by us. Considering our experience, we believe that the absorption test gives most valuable evidence, and much more than does the quantitative test, as to the identity or lack of identity between the bacterium used in immunization and the others tested against its specific serum.

<sup>1</sup>*Wien. klin. Wchnschr.*, 1903, 16, p. 691.



A difficulty frequently met with among recently isolated cultures is their lack of sensitiveness to agglutinins. This is probably due to their growth in blood or in fluid which has been derived from the blood. It is known that the growing of bacteria in a specific serum, and to some extent in any serum, lessens their agglutinability. Thus we cultivated the maltose-fermenting paradysentery bacillus (Flexner, Manila), on each of 11 consecutive days, in fresh broth solutions of the serum from a horse immunized through oft-repeated injections of the bacillus. The solutions used were 1½, 4, and 15 per cent. The serum agglutinated the culture before its treatment in dilutions up to 1:800. After the 11 transfers, the culture grown in the 15 per cent solution ceased to be agglutinated by the serum, and ceased to absorb its specific agglutinins. The cultures grown in the 1½ and 4 per cent solutions agglutinated well in dilutions up to 60 and 100, and continued to absorb agglutinins. The recovery of the capacity to be agglutinated was very slow when the culture was from time to time transplanted on nutrient agar.

It seems that, growing in serum dilutions, the bacteria which developed the least agglutinable substance were least hindered in their growth, and so developed most rapidly. Those producing the least agglutinable substance were thus finally the only ones surviving.

It is sometimes difficult to tell whether a culture is non-agglutinable or simply does not agglutinate in the serum used. An absolute test is to immunize an animal with it and see if it agglutinates in the serum.

THE DEGREE TO WHICH IT IS POSSIBLE TO DETECT THE MICRO-  
ORGANISM EXCITING A DISEASE BY THE SERUM REACTION  
OF THE BLOOD OF THE INFECTED PERSON.

The success of the Gruber-Widal test in suspected typhoid fever, cholera, and a few other diseases has given most persons an exaggerated opinion of the diagnostic value of a serum reaction. Even in these diseases the information given by the serum test is not so specific as is thought by many. The serum from typhoid patients occasionally agglutinates one of the varieties of the paratyphoid bacilli in higher dilutions than the typhoid bacilli. In 30 cases tested by us this happened in two instances. Grünberg and Rolly<sup>1</sup>

<sup>1</sup> *Münch. med. Wchnschr.*, 1905, 52, p. 105.

report the remarkable finding, in 40 cases of typhoid fever in which the typhoid bacilli were obtained, that in 35 per cent of the cases, the serum agglutinated a paratyphoid bacillus in higher dilutions than the typhoid bacillus. In these cases it is probably group-agglutinins, excited by the products of certain colon bacilli secondarily infecting the Peyer's patches, which agglutinated the paratyphoids, rather than the group agglutinins due to the typhoid bacilli.

As the clinician, when considering a case of continued fever, is as a rule trying to settle whether it is one of tuberculosis, malaria, or typhoid fever, he is satisfied to know whether the infection is or is not due to one of the typhoid-colon group, and does not mind the impossibility of an absolute identification of the variety. In the case of dysentery, a quantitative agglutination test is frequently useless as an indication whether the dysentery or paradysentery bacilli are exciting the disease. In cases due to the Shiga bacillus, the serum occasionally agglutinates one of the mannite-fermenting dysentery types in higher dilutions than itself. This again is probably due to group agglutinins produced by the absorption of substances contained in certain varieties of colon bacilli. A goat injected by us with a colon bacillus produced a serum which agglutinated it in a 1:5,000 dilution, and agglutinated the paradysentery bacilli in a dilution as high as 1:2,500. Agglutination of the Shiga bacilli by a serum which does not agglutinate the paradysentery bacilli usually indicates infection with the former variety, but an agglutination of the paradysentery bacilli alone may indicate a colon infection. Different members of groups of bacteria, like the colon group or the pneumococci, though having common pathogenic properties, frequently differ almost absolutely in their reaction to agglutinins. Thus, a sheep injected by us with a typical pneumococcus agglutinated that organism in a 1:100 dilution but did not agglutinate 20 other pneumococci in dilutions higher than 1:2. An equal variation was found by us to exist among the members of the colon group of bacilli. In infections which may be due to any one of a number of varieties differing in their agglutination characteristics, it is almost impossible to use sufficient cultures to diagnose by the serum reaction whether one of the group was the exciting factor.

The greatest limitation to the use of the serum reaction is the fact that the majority of bacteria do not, in the course of an infection, excite a sufficient amount of agglutinin to be readily detected, as for instance in the case of tubercle, influenza, and diphtheria bacilli.

Bacteria widely separated may, in exciting great quantities of agglutinin for themselves, develop so much group agglutinin for each other as to be misleading. An animal injected with staphylococcus agglutinated the typhoid bacillus in 1:160, while before, only 1:10. Another, injected with *B. proteus*, agglutinated a culture of this in 1:160,000, and also the typhoid bacillus in 1:1,200. In such a case, if the typhoid bacillus was suspected as the cause of the infection as above tested, the serum reaction would be apt to deceive.

In actual natural infections such very high reactions are improbable, but those sufficiently high to give misleading group reactions frequently occur. In adults the blood is apt to contain a considerable amount of group agglutinins for many bacteria before the special infection which is to be investigated developed. It is only through long experience that we are able to determine in how high dilutions such agglutinins are apt to act, and therefore in what dilutions a specific reaction can be suspected or considered proven. In suspected typhoid infection, for instance, we are now able to state that a reaction in a 1:50 dilution in two hours at room temperature is proof of an infection with a member of the typhoid-colon group, and as the great majority of such infections are due to the typhoid bacillus, we can consider this as the probable microorganism. Agglutination of the typhoid bacillus in higher dilutions makes this probability almost, but not quite, a certainty.

COMPARATIVE STUDIES OF DIPLOCOCCI DECOLOR-  
IZED BY GRAM'S METHOD, OBTAINED FROM  
THE SPINAL FLUID AND FROM THE NARES  
OF CASES OF EPIDEMIC CEREBRO-  
SPINAL MENINGITIS.

EDWARD K. DUNHAM.

THE studies which are here epitomized were undertaken as a part of the work of the Commission for the Study of Cerebrospinal Meningitis appointed by the Department of Health of the City of New York in March, 1905. The Commission deemed it important to ascertain whether microorganisms identical with those occurring in the cerebrospinal cavity were to be found in the upper air passages of those suffering from epidemic meningitis; and a part of the cultural work for the Commission was the isolation, from the nares of such cases, of diplococci not readily distinguishable from the diplococcus of meningitis by morphological characters.

Pure cultures of the *Diplococcus intracellularis* (Weichselbaum) were secured from the spinal fluid obtained by lumbar puncture from 50 cases of epidemic cerebrospinal meningitis and served for comparison with the cultures from the nares.

CULTURES.

The organisms from the nose or throat were obtained from streak-plate cultures made from the secretions collected with sterile cotton swabs. The medium employed for these plate cultures was 2 per cent glucose agar, enriched with ascitic fluid or sheep serum mixed with the melted agar at the time the plates were poured, or by distributing a little human or rabbit blood upon the surface of the agar after it had solidified. Cultures from the spinal fluid were obtained upon similar agar plates. Subcultures were made upon slants of 2 per cent glucose agar with or without other addition, or with a little blood or sheep serum upon the surface. The cultures on 2 per cent glucose agar without enrichment have usually exhibited excellent growth, provided the medium was protected from drying. Even the



slight drying due to loose cotton plugs has greatly impaired the growth; but when the plugs were tight, so that the surface of the medium was kept in a moist atmosphere, abundant growth could almost always be secured, and the cultures remained alive for a comparatively long time. To this end the air in the thermostat was also kept moist by placing a dish of water on the lowest shelf.

The cultures from spinal fluids upon 2 per cent glucose agar are gray and moist, usually only slightly raised above the surface of the medium, and appear to be strictly confined to those portions of the medium that were directly inoculated, there being no tendency to spread upon its surface. The condensation water becomes cloudy, with the formation of an abundant sediment, and, frequently, a slight pellicle is perceptible upon the surface of the condensation water. In cultures that are three or four days old the growth has a slight mucinous consistency, but is easily removed from the surface of the agar with a platinum loop. When scraped from the agar, the mass of bacteria has a pinkish buff color, and is readily broken up and disseminated in water or saline solution. A considerable variation in the luxuriance of growth was noted in many of the subcultures from different spinal fluids. In some cases a much more abundant inoculation of fresh media was necessary for success than in others, and the resulting growth was in the form of discrete colonies rather than a uniform layer. In other cases the growth was so abundant and formed such a thick layer upon the media as to excite the suspicion that the cultures were contaminated. These variations in growth depend not merely upon the particular strains under observation, but also upon the media employed. Slight indeterminate variations in the latter affected the luxuriance of growth very markedly. In two or three instances a particular lot of glucose agar was found to be unfit for use, even an abundant inoculation yielding no growth, although the preparation of the media could not have differed greatly from that employed when the result was most favorable.

An explanation of these experiences cannot be given; the method of preparing the medium had become an established routine from which there were not conscious departures.

Owing to the variations noted in the cultures on 2 per cent glucose agar, it is impossible to formulate a typical description of the growth

of the *D. intracellularis* on this medium that would serve for its certain identification.

Notwithstanding this fact, the cultures of diplococci decolorized by Gram's method of staining and obtained from the upper air passages could, in many instances, be readily distinguished from *D. intracellularis* when grown on 2 per cent glucose agar. These easily recognized as differing from this diplococcus were divisible into three groups:

1. Those yielding very luxuriant, heavy, mucinous growths of a gray color and forming a much thicker layer upon the agar than those of *D. intracellularis*. These cultures readily disseminated in saline solution and yielded a good suspension upon filtration through paper. They failed, however, to agglutinate in very low dilutions (1:20 or 1:50) of serum from a horse immunized with *D. intracellularis* from spinal fluids, while the latter was agglutinated in dilutions of 1:500 to 1:1000.

2. Those giving rise to very coherent growths that adhered strongly to the surface of the agar, so that it was extremely difficult to remove any considerable quantity of the growth with a platinum loop. This growth did not disseminate easily in saline solutions and was almost entirely removed by filtration through filter paper, so that satisfactory suspensions could not be obtained for agglutination tests.

3. Cultures which, within one to three days, produced a greenish-yellow fluorescence in the agar.

After eliminating these three groups, there remained:

4. A group of cultures which could not be readily distinguished by the gross appearances of the growth on 2 per cent glucose agar from cultures of *D. intracellularis* on the same medium. This group was of greater interest in the present inquiry than the other three and received most study. It could be divided into two subgroups: those more closely resembling *Micrococcus catarrhalis*, and those more closely resembling, if not identical with, *D. intracellularis*. These two subgroups may be provisionally designated as *catarrhalis*-like and *intracellularis*-like groups. The former were certainly not all of the same species, and it is doubtful whether any of them were identical with *M. catarrhalis* of Ghon and Pfeiffer. The *intracellularis*-

like group of diplococci appeared to be quite homogeneous, and there is reason to believe that they were identical with *D. intracellularis* of Weichselbaum. Coverglass preparations of the catarrhalis-like diplococci show somewhat less variations in size, form, and intensity of stain than similar preparations of *D. intracellularis* from spinal fluids, but these differences do not appear marked enough to inspire confidence in them as a means of distinguishing with certainty these diplococci from *D. intracellularis*. There was, however, a difference in the suspensions in 0.75 per cent solutions of salt. The organisms from the cultures of catarrhalis-like cocci showed a distinct tendency to settle and form a sediment within a few hours (although they readily passed through filter paper), while those of the intracellularis-like cocci remained in suspension for a very much longer time. This difference was constant. Within 24 hours a suspension of the growth from an agar culture of the former, one day old and containing enough organisms to render it distinctly opalescent, would yield a marked sediment when kept at rest over night in one of the tubes used for macroscopic agglutinations. Suspensions of *D. intracellularis* of like density failed to exhibit such sedimentation. In one instance such a suspension was kept in the ice-box for three weeks without more than a trifling sedimentation. These differences rendered macroscopic agglutinations of the catarrhalis-like group, for purposes of comparison, extremely difficult. Further differences, revealed by the readiness with which sera containing catarrhalis-like or intracellularis cocci could be filtered through Berkefeld filters, will be referred to later.

#### AGGLUTINATIONS.

Comparison of the various cultures of the fourth group and *D. intracellularis* was attempted with the use of macroscopic agglutination tests. For each test 1 c.c. of the serum properly diluted was mixed with 1 c.c. of a suspension of the organism in 0.75 per cent salt solution. In nearly all of these studies the final dilutions of the sera in each series of tests were: 1:20, 1:50, 1:100, 1:200, 1:1000, and 1:2,000. In a few cases higher dilutions were made, but did not add to the value of the results. The mixtures of serum, saline solution, and bacterial suspensions were placed in the incubator (37°) for three hours and then in the ice-box (about 5°) over night. At

the end of three or even six hours there was usually no well-marked evidence of agglutination, but after 24 hours the lowest dilutions showed distinct clumping. The first routine observations were made after from 15 to 24 hours, and a second observation from 42 to 48 hours after the tests were placed in the incubator. In the majority of instances the second observation revealed agglutination in one or two of the dilutions next higher than those giving evidence of this reaction at the first observation. The time which was allowed to elapse before making the observations is much longer than the usual limit adhered to in making macroscopical tests on agglutination. But it is believed that no errors due to growth of the organisms could vitiate the results. It is certain, from experiments made to determine this fact, that much more rapid reactions might have been secured if broth cultures of *D. intracellularis* had been used instead of suspensions from agar cultures. The saline suspensions were preferred, because it was believed that more uniform results could in this way be obtained in making tests at different times with an organism so capricious in vigor of growth as is *intracellularis*. Suspensions of apparently identical richness could be prepared without great difficulty at any time. The delay in reaction appeared to be relatively unimportant. In some of the tests  $\frac{1}{4}$  per cent of carbolic acid was added to the salt solution to preclude growth of the organisms. This addition did not in any way modify the results.

The sera employed in these agglutination tests were either from patients suffering from epidemic cerebrospinal meningitis or convalescent from this disease, or from animals which had been immunized with either single or repeated injections of large quantities of *D. intracellularis* obtained from spinal fluids. The animals used were rabbits, a goat, a horse, and several geese. The animals usually bore the injections of cultures from spinal fluids well. The most susceptible were the rabbits. But these soon acquired a considerable tolerance for even very large quantities, manifesting very slight, if any, acute symptoms, though they gradually became greatly emaciated, and lost much hair when the injections were continued for a long time. The geese were very tolerant of *D. intracellularis*. In one case about 5 c.c. of a thick purée prepared from 14 plate cultures (each about 10 inches in diameter) incubated for 24 hours,



was injected into the pectoral muscles of a goose without marked effect either locally or in the general condition. The animal refused food for a few hours, but on the next day ate as usual. The cultures of catarrhalis-like cocci and other diplococci, certainly not meningococci, from the nose and throat were often much more virulent for geese and rabbits when given subcutaneously or intraperitoneally, the first injection frequently causing death.

The goat was immunized by Dr. Simon Flexner, who kindly furnished the serum for these tests. Large quantities of *D. intracellularis* from the spinal fluids obtained from 13 cases of epidemic meningitis were used by him to immunize this animal, and the injections were repeatedly given during a period of many weeks.

The horse serum came from an animal immunized with large quantities of dead cultures at the Research Laboratory of the New York Department of Health, and was generously contributed by Dr. Park. Some of the sera were preserved with chloroform, trikresol, toluol, or  $\frac{1}{4}$  per cent phenol; others received no preservative, but were collected and kept uncontaminated. Comparative tests failed to reveal any influence exerted by these additions upon the agglutinating power of the sera.

None of these sera showed a high specificity in their agglutinations of *D. intracellularis*. They agglutinated in dilutions from 1:200 to 1:4,000, or two to ten times as great as the sera from normal animals of the same species. But it is difficult to utilize even this moderate increase in agglutinating power, because the conditions under which the tests were made differed unavoidably at different times, and even slight variations in condition influence the limits of the dilution at which positive results are obtained in a very great degree. The density of the emulsion, for example, was found to make a very great difference in the development of distinct agglutination in the higher dilutions. This fact is probably related to the observation that a complete agglutination of all the organisms in an emulsion, with a sedimentation of the resulting clumps, rarely takes place when the macroscopic method and saline suspensions are used. Free organisms are almost always present in sufficient abundance to render the fluid perceptibly opalescent, even when the agglutination of the remainder of the organisms gives rise to relatively large and

heavy clumps that settle to the bottom of the liquid. Complete agglutinations are, therefore, comparatively rare when this method is employed. They appear to be much more common, especially in the lower dilutions, when broth cultures are used in place of suspensions in normal saline solution.

The fact, already mentioned, that the suspensions of catarrhalis-like diplococci tended to form a distinct sediment within 24 hours, rendered a comparison of the agglutinating power of the sera upon this group of organisms with the agglutination of intracellularis cocci practically impossible, but this sedimentation itself furnished a means of distinguishing these cultures from those of the latter organism. The difference between an agglutination with settling of the clumps and a sedimentation of the individual organisms is readily demonstrable upon moderate amplification. For this purpose a microscope with the tube in a horizontal position and a Leitz No. 3 objective were used to control the macroscopic observations. By rotating and then suddenly tilting the agglutination tube the sediment could be thrown up from the bottom along the side of the tube and readily examined with the microscope. The latter was placed in front of a window and the mirror removed. A very small diaphragm was employed, and the individual bacteria could be distinguished without difficulty. This microscopical control, therefore, served also to eliminate contamination with organisms morphologically differing in marked degree from *D. intracellularis* which might occasion errors in the observations.

The foregoing observations suffice to show the futility of an attempt to use these methods of agglutination in the comparative study of *all* the diplococci decolorized by Gram's stain found in the nose and throat, and those obtained from spinal fluids. In some cases they simply demonstrate constant differences in the suspensions of different cultures.

It was thought that more obviously biological differences or likenesses of general applicability might be revealed if the various agglutinins in the sera were removed by absorption with large quantities of a given species, and the serum thus deprived of one set of agglutinins applied to suspensions of the various organisms under study,

with a view to determining which of these species were then agglutinated. Many such experiments were made, with apparently gratifying success, but a critical review of the results casts much doubt upon their significance and also reveals unexpected difficulties in technique, which have not yet been overcome.

It was found that *D. intracellularis* from spinal fluids apparently removed all the agglutinins capable of clumping the organisms derived from the spinal fluids and also, as far as could be determined, those agglutinating the catarrhalis-like organism and other members of the fourth group from the nares; whereas the latter failed to remove from the serum of animals immunized with *D. intracellularis* more than a fraction of the agglutinins clumping *D. intracellularis*. But the very heavy suspensions necessarily used to insure absorption of the agglutinins were very different in the two cases. It was comparatively easy to separate the catarrhalis-like organisms from the diluted serum (usually diluted 1 in 5 or 1 in 10 with normal salt solution) either by centrifugalizing or by filtration through a Berkefeld filter. But it was found to be impossible to free the serum sufficiently from *D. intracellularis* with any available centrifuge. Although most of the organisms were thrown down, so large a number remained in suspension that the use of the serum was very unsatisfactory, especially in view of the delayed reactions already described. The separation of the serum by filtration through a Berkefeld filter, while successful, was exceedingly slow in comparison with the similar filtration when cultures of the catarrhalis-like cocci were employed. The heavy suspensions of *D. intracellularis* quickly formed a dense coating upon the surface of the filter, while an equally dense suspension of the catarrhalis-like organisms was more granular, or less slimy, and the deposit upon the filter impeded the passage of the serum in a very much less degree. This difference in the physical character of the two suspensions, which persisted in considerable, though less degree, even when the centrifuge was used before filtration, excited the suspicion that the agglutinins of the serum were mechanically held back by the coating of organisms or slimy substances in the suspension, and this suspicion was increased by the observation that a heavy suspension in diluted immune serum

of a culture from the throat, which was certainly neither *D. intracellularis* nor one of the catarrhalis-like group, but which produced much mucinous material on 2 per cent glucose agar, also filtered exceedingly slowly and removed nearly if not quite all the agglutinins clumping *D. intracellularis* and intracellularis-like diplococci. If the loss of agglutinins were due to a mechanical prevention of their passage through the clogged filter and not to a union with the organisms, the value of these absorption tests, when all the agglutinins have been removed, is so seriously impaired as to cast doubt on their having any specific value, though they do reveal differences in the cultures used for absorption and, for this reason, are not without significance.

To test the effect of clogging a Berkefeld filter with a colloid so as to lengthen the time required for the passage of a diluted serum, a 1 per cent colloidal solution of corn starch boiled in 0.75 per cent salt solution was used as a diluent of the immune serum, and compared with a similar dilution with normal salt solution used as a control. The latter passed through a Berkefeld filter in 15 minutes. The starch mixture required about 20 hours, or 80 times as long. The agglutinating limits of the starch filtrate upon various of the organisms under study was found to be only  $\frac{1}{4}$  to  $\frac{1}{10}$  that of the control. This result appears to support the idea that the agglutinins might be mechanically held back by the coating upon the filter when intracellularis was used for absorption. It is possible, however, that the colloidal starch may have had some direct action upon the agglutinins in the serum. But this might also be the case with colloidal (slimy) substances in the cultures of *D. intracellularis*. That the agglutinins may in this case be mechanically separated by filtration is also suggested by an observation indicating that the removal of the major part of the diplococci from the diluted serum with the centrifuge somewhat hastens subsequent filtration, but furnishes a filtrate with greater agglutinating power than it possessed when filtration was slower.

Notwithstanding the fact that these absorption tests have failed to establish a definite union between specific agglutinins in the immune sera and the intracellularis organisms, they throw some light on the



relations between *D. intracellularis* and the diplococci of the fourth group found in the nose and throat. Some of the latter were identical in behavior with *D. intracellularis*; others yielded suspensions much more readily filtered or freed from the organisms with the centrifuge.

#### FERMENTATION.

A more striking biological distinction between *D. intracellularis* and the catarrhalis-like group of cultures was the differences they exhibited in the production of acid when grown in a suitable medium containing dextrose. Twenty-six of the cultures from the spinal fluid, three from the blood, and sixteen from the nose or throat of patients with epidemic meningitis were studied with a view to determining the production of acid when grown in the presence of dextrose.

The medium employed was a mixture of nutrient broth containing 1 per cent dextrose, sheep serum diluted with three times its volume of water, and 1 per cent solution of Kahlbaum's or Merck's purified litmus. These three constituents were separately sterilized in the Arnold sterilizer on three successive days, and then one part of the diluted serum mixed with three parts of the broth and enough of the litmus solution added to impart a distinct color to the whole. The mixture thus prepared was incubated for two to three days to detect accidental contamination. All of the cultures of *D. intracellularis* from spinal fluids and all of the cultures from the blood produced an unmistakable acid reaction in this medium after incubation at 37° for 24 hours. The intensity of this reaction usually increased slightly during the next 24 to 48 hours, but in no case was the acid production sufficiently abundant to occasion coagulation in the medium. When the reaction has reached a certain degree of acidity it appears to remain about constant, and to render the medium unfavorable for the further growth of the organisms. At the end of nine days only three out of 29 such cultures were found to contain living organisms. Similar cultures made with the organisms from the nose and throat differentiated them into three groups: (1) those which produced acidity without coagulation, i. e., the same change wrought by *D. intracellularis*; (2) those causing the formation of acid with coagula-

tion in 24 hours; and (3) those producing no acidity (or, perhaps causing an alkaline change of reaction) even after nine days' incubation.

Of the 16 cultures from the nose and throat, six affected this broth-serum-glucose medium in exactly the same way as *D. intracellularis*. Of these six cultures a few had already been used for observations concerning their susceptibility to agglutination and their ability to absorb agglutinins from immune sera. In all these respects and in the characters of the suspensions they furnished, and in their various cultures, they corresponded to parallel observations on *D. intracellularis*. As far as our knowledge can justify a conclusion, these cultures were identical with this diplococcus and the latter were, therefore, present in the upper air passages in the cases of epidemic meningitis from which these cultures were obtained.

Most of the work furnishing the results here summarized was done during the summer of 1905 with the assistance of Mr. Hubert C. Ward, to whom grateful acknowledgment for many helpful suggestions and unflagging interest is cordially extended.

# THE FREQUENT OCCURRENCE OF MENINGOCOCCI IN THE NASAL CAVITIES OF MENINGITIS PATIENTS AND OF THOSE IN DIRECT CONTACT WITH THEM.\*

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EVERYONE familiar with the investigations concerning the etiology of meningitis knows that, owing partly to the difficulty of isolating and keeping alive the meningococcus, partly to its similarity to other micrococci, the work of most investigators has been incomplete and therefore of little permanent value.

As the amount of influence which the results of the investigations here recorded may exert depends largely on the degree to which others are convinced of the thoroughness of the identification of the organisms found in the nasal cavities, it seems best to review briefly the literature in order to see what characteristics the best observers consider as belonging to the meningococcus and therefore as necessary to prove the identity of the suspected organism.

The first important study of the etiology of primary cerebro-spinal meningitis was undertaken by Weichselbaum in 1887. Before that time it had been pretty well established that in secondary cases the pneumococcus was at times the exciting factor, though Leyden<sup>1</sup> and Leichtenstern<sup>2</sup> had noted diplococci in the exudate of fatal cases of primary cerebro-spinal meningitis which they believed to be different from pneumococci. Their descriptions lead one to think that they really saw the meningococcus, but their work was too meager to establish this.

In 1887 Weichselbaum<sup>3</sup> isolated, and carefully studied, cultures from six typical cases of cerebro-spinal meningitis. The cocci had the following cultural characteristics. They grew well on nutrient agar-agar containing 2 per cent of gelatin. The growth on the surface was rather flat and viscid; it was gray in direct, and grayish-white in transmitted, light. The borders were indented and showed the growth to be made up of confluent colonies. Potato showed no visible growth. On the agar-gelatin plates the deep colonies were very small. The surface colonies were grayish white. Under the microscope they were round or irregular, finely granular and their borders indented. They had a golden brown nucleus, an inner light yellow zone, and an outer one which was transparent and colorless. Weichselbaum found it neces-

\* Technical portion of Part I of an investigation of cerebro-spinal meningitis carried on under the auspices of the Special Commission of the Department of Health of New York City.

sary to transplant the cultures every two days in order to keep them alive, as they were found to die usually in from three to six days. The cocci themselves were mostly in pairs; some were single and a few in tetrads and small heaps. The single cocci were round, the pairs flattened at the apposed ends. The cocci varied greatly in size and staining, the larger forms, which stained more deeply, being sometimes twice as large as the smaller, more faintly staining ones. They were mostly intracellular in the exudate, and were found only in small numbers in the tissues. All the cultures were Gram negative, and grew well only at blood heat. They did not grow at all at 20° C.

In 1895 H. Jaeger<sup>4</sup> published the results of the study of 14 cultures isolated from typical epidemic cerebro-spinal meningitis. The organisms he isolated differed from those of Weichselbaum and more recent investigators in the following characteristics. There were short chains of four to six elements present in all the cultures, and in two cultures there were long chains of 20 to 30. He describes the cultures as being sometimes Gram positive and sometimes negative. However, he never found Gram positive cocci in the tissues. His cultures grew at lower temperature. The viability of his cultures was much greater, one culture in broth living 43 days. The culture stood drying for 96 days and pus dried on linen gave a growth of the cocci after 127 days. A capsule was present in the smears.

A. Heubner,<sup>5</sup> Jaeger's strongest supporter, describes cultures from four cases which were identical with Jaeger's. Jaeger in his article of 1899<sup>6</sup> still clings to his description of 1895. In 1901<sup>7</sup> he decides that the meningococcus has no capsule, but in other points holds to his original position; nor does he add anything new in his paper of 1903.<sup>12</sup>

Between Jaeger's first and last papers a series of investigations had been carried on which demonstrated to most bacteriologists that either he had failed to isolate the true organisms exciting the disease, or had allowed contaminating or associated bacteria to overgrow and displace the meningococci in his cultures.

Councilman, Mallory, and Wright,<sup>8</sup> after a thorough study of 31 cases, describe their cultures as being similar to those of Weichselbaum.

In 1901 Albrecht and Ghon,<sup>9</sup> after working with 22 cultures, agreed with Weichselbaum. The greater number of cultures observed led them to give wider limits of temperature as suitable for development. Some cultures grew from 25°-40°, though the maximum growth was always between 36°-37°. They are the first to describe the "bread crumb" granules found in the center of the colony after 48 hours. They give the best media as Loeffler's blood serum, or agar containing ascitic fluid. A pellicle on the broth cultures, when the broth was neutral and the cultures were left quiet for several days, was almost constant. In a few instances they kept cultures alive, when protected from drying, for 185 days without transplanting. All the cultures were Gram negative and there was no tendency to chain formation. Albrecht and Ghon obtained cultures from Jaeger and from Heubner and found them not only quite different from theirs, but also unlike each other.

Albrecht and Ghon,<sup>10</sup> and Weichselbaum,<sup>11</sup> in convincing articles published in 1903, take up the peculiarity of Jaeger's cultures point by point, and are of the opinion that he was not working with true meningococcus cultures. Taking the important points agreed upon by the best workers, Albrecht and Ghon give the following characteristics as essential in identifying true meningococcus cultures.

1. Gonococcus-like in form, dividing in the same way, always Gram negative, having many degeneration forms, and often intracellular.



2. Growing only at fairly high temperature,  $25^{\circ}$ - $42^{\circ}$ , maximum growth at  $36^{\circ}$ - $37^{\circ}$ .
3. Colonies on agar plates luxuriant, quite viscid, glistening, gray in direct light and grayish-white in transmitted light.
4. Growth confined almost entirely to surface in stab culture.
5. Develops pellicle on broth culture (when the broth is neutral and the cultures are undisturbed for several days).
6. Slight pathogenicity for ordinary animals.
7. Non-resistant.

#### MICROCOCCUS CATARRHALIS.

In 1901 Ghon and H. Pfeiffer<sup>13</sup> published the results of the study of 40 cultures of *Micrococcus catarrhalis*. They found that, while it grew best on blood agar, it would grow on ordinary media. It differed from the meningococcus in growing more easily, more luxuriantly, and at a lower temperature. The colonies under the microscope were darker, more compact, and had more abrupt margins. Jaeger<sup>14</sup> finds all the strains of *M. catarrhalis* self-agglutinating. Some of the cultures examined by us have had all the above characteristics, while others have more nearly resembled the meningococcus.

#### MENINGOCOCCUS CULTURES ISOLATED BY PREVIOUS INVESTIGATORS FROM THE NASAL MUCUS.

In going over the literature we are impressed with the small number of cases from which thoroughly identified meningococcus cultures have been isolated from the nasal mucus. The cases from which Gram negative diplococci closely resembling meningococci have been found in the smears from the nose and throat are, on the contrary, numerous, and have been found by nearly all workers on meningitis.

The first to identify as meningococcus a culture taken from the nasal mucus was F. Kiefer.<sup>15</sup> While working with meningitis cultures he developed a severe purulent rhinitis. The pus contained numerous meningococci. In 1898 Schiff<sup>16</sup> isolated cultures from three out of 29 dispensary patients, a portion of whom suffered from chronic laryngitis, which cultures, he says, Weichselbaum considered true meningococci. These three cultures will be considered later in connection with two obtained by us from medical students, which agreed with the meningococci obtained from the spinal fluid in all respects except in agglutination characteristics. Councilman, Mallory, and Wright<sup>8</sup> report one culture from the throat of a tonsilitis case. Griffon and Gandy<sup>17</sup> twice, at an interval of five days, isolated cultures from the nose of a meningitis case which were identical with cultures from the spinal fluid. Albrecht and Ghon report two instances, one from a case of meningitis, the other from a man whose child died of meningitis three days before the culture was taken. F. Lord,<sup>18</sup> of Boston, isolated meningococci from a case of rhinitis. A. Weichselbaum and Ghon<sup>19</sup> identified one culture from the nose of a meningitis patient and three from the noses of people in contact with patients. These cultures from the 14 cases were the only ones we could find that were studied with sufficient care to warrant their acceptance as true meningococci.

## ORIGINAL INVESTIGATION.

Most of the material for this investigation was obtained through the courtesy of Dr. A. W. Taves, of Gouverneur Hospital.

The mucus was taken from the nasal fossæ with a sterile cotton swab and plated out as soon as possible on ascitic agar. As a rule the plates were made within one hour of collecting the specimen, while the swab was still moist. These plates were incubated for from 24 to 48 hours, then fished in the usual way. The colonies were put on blood agar, which seemed to be the most favorable medium.

Several colonies were fished from every type found which resembled a meningococcus colony in color or granularity, and which, under the high power, showed diplococci resembling meningococci. The organisms from the cultures were stained by Gram, and several of the Gram negative ones, which in cultures resembled meningococci, were kept for study.

Fifty-two meningitis cases were examined. Meningococci were isolated from 12 of the 22 cases examined during the first week of the disease, and from 5 of the 15 examined during the second week. In six cases examined during the third week, three during the fourth, and six between the fifth and ninth weeks, no meningococci were found, while in a very severe case examined on the 67th day, we found a few colonies. In one case we failed to get them on the first day and found them in large numbers on the second.

From this it would seem as though the meningococci were present in a rather large percentage of the cases during the first week of the disease.

The nasal secretions of 45 healthy persons living in close contact with meningitis patients were examined. In five of these, meningococci were isolated during the first two weeks of the patients' illness. From the nasal mucus of 55 first year medical students who had never been in known contact with meningitis, there were isolated in two cases a few organisms which were, culturally and in pathogenicity, like meningococci. In studying their agglutination, however, we found that they differed from our other cultures in their specific agglutinins, and therefore were differentiated in one important respect from the latter. In this connection it is of interest that Schiff, in describing his cultures from the nasal cavity of people

not in contact with meningitis, does not refer to agglutination, and evidently did not make the test. His cultures may have differed as ours do. One cannot safely classify these atypical cultures. They may be meningococci derived from a strain different from those isolated by us in the present epidemic, or organisms not capable of readily exciting meningitis, and yet so closely related that they cannot be differentiated without more careful cultural tests than we at present use.

The following tables give the cases, the day of the disease when the specimen was taken, the termination of the disease, and the bacteriological findings.

TABLE 1.

CASES OF MENINGITIS IN WHICH MENINGOCOCCI WERE ISOLATED FROM THE NASAL MUCUS.

Name	Day of Disease	Termination	Percentage of Meningococcus Colonies Present in Plates
W. W.....	1	Died	About 55
J. N.....	2	Died 3d day	" 90
L. Z.....	3	" ?	" 30
E. R.....	3	Died 4th day	" 50
R. T.....	3	" ?	" 40
J. G.....	4	" ?	" 95
Mrs. M.....	4	Died 6th day	Very few
S. F.....	5	Died	About 50
S. K.....	5	" ?	" 90
J. S.....	5	" ?	" 30
D. M.....	7	Recovered	" 2
C. P.....	7	" ?	Few
M.....	10	Died	About 10
M. G.....	10	"	" 2
J. M.....	12	"	A very few
M. H.....	14	" ?	About 95
E. S.....	14	Died	" 5
S. K.....	67	Died 60th day	" 2

TABLE 2.

CONTACTS WITH MENINGITIS CASES FROM WHOM MENINGOCOCCI WERE ISOLATED FROM THE NASAL MUCUS.

Name	Day of Patients' illness	Time Since Last Contact	Condition	Percentage of Meningococcus Colonies Present in Plates
Mr. D.....	.....	4 days	Normal	About 95
Mrs. D.....	.....	4	"	" 95
Mrs. K.....	14th day	Still in contact	"	" 95
A. K.....	14th "	" "	"	" 50
Mrs. M.....	.....	14 days	"	" 30

The plate cultures from the mucus of all these cases contained many colonies and in most cases great numbers of colonies.

TABLE 3.

CASES OF MENINGITIS IN WHICH MENINGOCOCCI WERE NOT ISOLATED FROM THE NASAL MUCUS.

No. Examined	Day of Disease	No. Examined	Day of Disease	No. Examined	Day of Disease
1.....	1	2.....	13	1.....	24
1.....	2	5.....	14	1.....	27
2.....	3	1.....	15	1.....	28
5.....	6	1.....	17	1.....	31
1.....	7	1.....	18	1.....	40
1.....	9	1.....	19	1.....	42
1.....	10	1.....	20	1.....	49
1.....	11	1.....	21	1.....	60

TABLE 4.

CONTACTS WITH MENINGITIS CASES FROM WHOM NO MENINGOCOCCI WERE ISOLATED FROM THE NASAL MUCUS.

Number Examined	Days since Contact	Number Examined	Days since Contact
5	2	1	35
9	3	1	50
2	4	3	56
1	10	1	60
1	18	16	Still in contact

All contacts were occupants of the same rooms and nearly always members of the family.

From 14 cases we took multiple specimens. In only one case did we find meningococci in two specimens, 90 per cent on the fifth day, and a very few on the tenth. Table 5 gives the cases, the day of disease, and the bacteriological findings.

#### CULTURAL CHARACTERISTICS OF THE MENINGOCOCCI ISOLATED FROM THE NASAL MUCUS.

The cultures isolated from the nasal mucus were carried out on the different laboratory media and compared with 30 cultures isolated from a similar number of specimens of spinal fluid.

There were no apparent differences between the nose and spinal fluid cultures. Some grew more luxuriantly than others. The more luxuriant cultures from both spinal fluid and nose seemed to have a more yellow tone, while those growing in a thinner layer were grayish-white.

The morphology of the organisms differed slightly, but the differences were the same for cultures from both sources.

The meningococci occurred as flattened cocci in pairs, fours, and sixes. They varied widely in size in the same culture from the same media, and differed greatly in the intensity with which they took the stain.

In no case did a culture tend to be Gram positive. Cultures were



repeatedly plated out, and numerous colonies fished and stained by Gram. In a culture transplanted twice a day for five days on Loeffler's blood serum, so that the organisms might all be very young; there was no tendency for any of them to be Gram positive.

TABLE 5.  
CASES OF MENINGITIS FROM WHICH MULTIPLE SPECIMENS WERE EXAMINED.

Name	Day of Disease	Termination	Findings
B. I.....	13	Recovery	No meningococci
	14		" "
	15		" "
	16		" "
E. E.....	2	Died 7th day	" "
	3		" "
G. D.....	7	Died 45th day	" "
	13		" "
	19		" "
K. S.....	5	?	90 per cent
	10		A few
	16		No meningococci
M. D.....	7	Recovered	2 per cent meningococci
	13		No meningococci
	19		" "
C. P.....	3	?	" "
	4		5 per cent meningococci
	5		No meningococci
	7		" "
	8		" "
S. M.....	9	?	" "
	2		" "
	6		" "
S. J.....	8	Recovered	" "
	6		" "
	7		" "
S. T.....	9	Died 10th day	" "
	10		" "
	3		" "
S. J.....	4	?	" "
	5		30 per cent meningococci
	10		No meningococci
W. F.....	18		" "
	3		" "
W. J.....	4	?	" "
	17		" "
	18		" "
	19		" "
	20		" "
Z. L.....	22		" "
	1		" "
	3		30 per cent meningococci

The method of staining by Gram was the same throughout the work and was briefly as follows:

1. Stain two minutes in anilin gentian violet
2. Displace anilin gentian violet with Gram's Iodine Solution and leave on one and one-half minutes.
3. Wash in 95 per cent alcohol until visible color stops coming out.
4. Wash in water and counter stain 30 seconds in watery solution of Bismarck brown (2 grams in 100 c.c.)

In no culture was any tendency to chain formation observed. The cultural characteristics of colonies on ascitic agar plates were as follows:

1. *Macroscopic appearance*.—In many cultures there are two distinct zones, but this was not found constant on repeated plating. Where the colonies are in contact, they are usually divided by a distinct line. They are oval or irregular, grayish-white to yellowish-white, moist and usually viscid, flowing about the needle instead of breaking away from it when they are fished.

2. *Microscopic appearance: Low power*.—Pale amber to brown in color. From fine and evenly granular colonies to those with very coarse central granules. Margins generally rather even and often not abrupt.

3. *Microscopic appearance: High power*.—The diplococci, and occasionally the fours, show plainly. On some plates the margins are smoother and more abrupt, and the separate organisms are distinguished with difficulty.

The most constant characteristics seem to be the coarse central granules and the characteristic separate organisms at the margins when observed with high power.

*Ascitic agar slants*.—Grayish-white, fairly luxuriant growth, usually with discrete colonies. These colonies at times have a diameter of five millimeters at 48 hours. They are generally quite round, but vary a good deal in the waviness of their outlines. Two zones are often distinguished. In the smears from ascitic agar the organisms stain poorly and are indistinct.

*Loeffler's blood serum*.—The growth is heavy, moist, confluent and yellowish. The smears show the organisms distinctly, and usually of larger size than on ascitic agar.

*Plain agar*.—Growth scant, if any, and generally consisting of a few isolated colonies.

*Glucose agar*.—Growth slightly better than on plain agar.

*Glycerin agar*.—Same as plain agar.

*Blood agar*.—Growth very luxuriant, confluent, yellowish-white and extremely sticky; smears same as from Loeffler's.

*Sheep serum agar*.—Growth fairly luxuriant, about the same as ascitic agar.

*Gelatin*.—No cultures grew below 24°. At 37° C. all the cultures grew well, with the formation of a heavy pellicle. At the end of six weeks the gelatin still hardened when put in the ice box.

*Hiss's inulin medium*.—Rendered opaque but not coagulated.

*Litmus milk*.—The cultures grew only slightly and turned the milk somewhat darker than control at the end of 48 hours, but made no further change.

*Marble broth*.—Most of the cultures grew slightly, a few grew well, making the medium cloudy, afterward forming a pellicle and sediment. The pellicle was quite general after one week.

*Plain broth*.—Very few cultures grew in our broth and these only slightly. This was possibly due to an unsuitable reaction of the broth.

*Dunham's peptone solution*.—Growth very slight. Indol not produced.

*Glucose litmus peptone sheep serum agar*.—Acid produced after 48 hours.

*Lactose litmus peptone sheep serum agar*.—Acid produced after 48 hours.

*Maltose litmus peptone sheep serum agar*.—Acid produced after 48 hours.

*Saccharose litmus peptone sheep serum agar*.—Acid not produced after 48 hours.

*Mannite litmus peptone sheep serum agar.*—Acid not produced after 48 hours.

*Temperature.*—The maximum growth was at about 37°. Nearly all the cultures grew at 30° three months after isolation; a few grew slightly at 24°.

*Viability.*—The cultures varied greatly in the length of time which they would live without transplanting. In order not to lose cultures we reinoculated them every five days. Many of the cultures on ascitic agar lived from 10 to 20 days without protection from drying, and some of the broth and gelatin cultures lived from five to eight weeks. After 25 cultures were kept in the ice box for five days none of them were alive. Cultures left at room temperature and in the ordinary amount of light varied greatly in their resistance. Most of them failed to grow after 48 hours.

#### AGGLUTINATION.

Weichselbaum and Ghon<sup>19</sup> and Bettencourt and França<sup>20</sup> found that the serum of meningitis patients agglutinated meningococci in from 1:10 to 1:100 dilutions. They found that the serum of animals immunized for a long time with meningococci agglutinated the cultures only in low dilutions, 1:100 being the highest. We tested the serum of very few patients. The highest dilution agglutinating was 1:200.

Finding it impossible to distinguish between nasal and cord cultures by morphological or cultural comparison, we have made use of a specific serum to aid in classifying the cultures from the different sources.

We inoculated two horses, two sheep, three goats, and 20 rabbits. Only two rabbits lived long enough to give a serum of sufficient agglutinating strength to help in our work. Of these two, one was inoculated with a nasal culture from a student not in contact with meningitis. This serum agglutinated its own culture and several typical meningococcus cultures completely in a dilution of 1:40. The other was inoculated with a cord culture, and agglutinated its own culture in a 1:400 dilution, and other cultures in a 1:50 or slightly higher dilutions.

One sheep, after being inoculated with rather large doses of a cord culture for over three months, gave a serum agglutinating most of the cultures completely in a 1:40 dilution. The goat sera never agglutinated above 1:20.

One horse was inoculated with a nasal culture obtained from a severe case of meningitis on the second day of the disease. The patient died on the third day. This horse died after a month's treatment, before the serum was of much value. The other horse

was given a cord culture, and though he became very sick at the end of the first month, he improved when given smaller doses. At the end of four months the agglutinating strength of this serum was 1:100 for most of our cultures. It seemed better for some other cultures than for its own.

There was a great difference in the degree of agglutinability of the cultures on different days, which made it very difficult to compare the results quantitatively.

The following tables give some of the serum tests with cultures from the spinal fluid and noses of patients, and from the noses of

TABLE 6.

AGGLUTINATION OF 22 CULTURES OBTAINED FROM THE SPINAL FLUID, AND OF 21 FROM THE NASAL MUCUS BY SERUM OF SHEEP 182 AFTER ANIMAL HAD BEEN INOCULATED FOR THREE MONTHS.

	Control	1:20	1:50	1:100	1:200	1:400
33-2 nose.....	—	+	++	+	+	—
XI-2 nose.....	—	+	++	+	—	—
124-1 cord.....	—	+	+	±	—	—
VII-3 nose.....	—	+	+	±	—	—
W. P. 1 cord.....	—	+	+	±	—	—
108-5 cord.....	—	+	+	±	—	—
D. Getz cord.....	—	+	+	±	—	—
Wiesbard cord.....	—	+	+	±	—	—
105-2 cord.....	—	+	+	±	—	—
95-2 nose.....	—	±	—	—	—	—
140-2 cord.....	—	+	±	±	—	—
91-1 nose.....	—	—	+	+	—	—
114-2 nose.....	—	+	±	—	—	—
152-1 cord.....	—	+	+	±	—	—
M142-2 nose.....	—	+	±	±	±	—
Pregalia cord.....	—	+	+	±	—	—
140-3 cord.....	—	—	—	—	—	—
Stolz-2 nose.....	—	+	±	—	—	—
Cohen cord.....	—	±	±	±	—	—
Fieland cord.....	—	+	+	+	—	—
Fieland nose.....	—	+	+	+	±	—
Goldfarb cord.....	—	+	+	+	—	—
Schwartz nose.....	—	+	+	+	—	—
Goldfarb nose.....	—	+	+	±	—	—
182 cord.....	—	++	++	—	—	—
Merritt nose.....	—	+	±	—	—	—
136 cord.....	—	+	+	+	—	—
23-2 nose.....	—	++	+	—	—	—
IX-2 nose.....	—	+	—	—	—	—
Horowitz cord.....	—	—	—	—	—	—
14 cord.....	—	++	—	—	—	—
105-5 nose.....	—	+	+	±	—	—
253-5 cord.....	—	+	+	—	—	—
36-8 nose.....	—	++	++	—	—	—
20-3 nose.....	—	+	+	+	±	—
25-1 nose.....	—	+	+	—	—	—
142-S cord.....	—	+	±	—	—	—
Bayridge cord.....	—	+	—	—	—	—
Rubin nose.....	—	±	+	±	—	—
Marzo nose.....	—	+	+	±	—	—
Fielder cord.....	—	+	+	+	—	—
Gruno cord.....	—	++	++	+	—	—
McDonald nose.....	—	±	+	—	—	—

In testing the agglutinating power we used emulsions made from 24 hour sheep serum agar slants in normal salt solution. We used hanging drops, with the slides inverted until the moment of examination, to prevent mistaking mechanical grouping for agglutination. The hanging drops were usually examined after four hours and marked in the following way: —=no agglutination, |=trace, ±=marked trace + = good agglutination + |=very good agglutination, and ++=complete agglutination.



contacts and from people not in contact. As a rule, the majority of the cultures seem to agglutinate as well as the culture with which the animal was inoculated.

We saturated the best horse serum with its own culture, with nasal cultures (*a*) from a severe meningitis case, (*b*) from a contact, (*c*) from a non-contact, and with several *M. catarrhalis* cultures. After allowing the mixture of serum and culture in a 1:5 dilution to stand

TABLE 7.

TESTS OF THE SERUM OF HORSE 277 AFTER BEING INOCULATED FOR FOUR MONTHS WITH 142 S, A SPINAL FLUID CULTURE.

CULTURES	SERUM		SERUM EXTRACTED WITH XI-2, A MENINGOCOCCUS CULTURE FROM THE NASAL MUCUS OF A MENINGITIS PATIENT			SERUM EXTRACTED WITH A <i>M. CATARRHALIS</i> CULTURE FROM A MENINGITIS CASE		
	1:20	1:40	1:5	1:10	1:20	1:5	1:10	1:20
Gruno cord.....	+	+	—	—	—	+	+	+
Fielder cord.....	+	+	—	—	—	+	+	+
142 S cord.....	+	+	—	—	—	+	+	+
XI-2 nose.....	+	+	—	—	—	+	+	+
33-2 nose.....	+	+	—	—	—	+	+	+
36-8 nose.....	±	±	—	—	—	+	—	—
W. nose.....	+	+	+	+	+	+	+	±

These sera after being extracted were in a 1:5 dilution filtered through a Berkefeld filter and the third 10 c.c. used.

for three hours, we filtered through a Berkefeld filter, and used the third 10 c.c. of the filtrate. All the meningococcus-like cultures seemed to remove the agglutinins for all the cultures, while the *M. catarrhalis* cultures only reduced them about one-third. The control filtration of the serum without exhaustion reduced the agglutinins about as much as did the *M. catarrhalis* cultures.

TABLE 8.

TESTS OF THE SERUM OF HORSE 277 AFTER BEING INOCULATED FOR FOUR MONTHS WITH 142 S, A SPINAL FLUID CULTURE.

CULTURES	SERUM UNEXTRACTED		SERUM EXTRACTED WITH A SPINAL FLUID CULTURE		SERUM EXTRACTED WITH W. n., A CULTURE FROM A PERSON NOT IN CONTACT WITH MENINGITIS		
	1:100	1:200	1:10	1:20	1:20	1:40	1:100
Gruno cord.....	+	—	—	—	+	—	—
Fielder cord.....	—	—	—	—	+	—	—
142 S cord.....	+	—	—	—	+	—	—
XI-2 nose.....	+	—	—	—	+	—	—
33-2 nose.....	+	—	—	—	+	—	—
36-8 nose.....	+	—	—	—	+	—	—
W. nose.....	+	—	—	—	—	—	—

The sera, after being extracted, were centrifuged instead of filtered.

We saturated this same horse serum with a meningococcus culture, with a *M. catarrhalis* culture, and with "W. n." from a student, a non-contact case. Instead of filtering we centrifuged, and found our results somewhat different. The meningococcus culture took out all the agglutinins for the meningitis culture and not for the others, while the *M. catarrhalis* and the "W. n." left in over half the agglutinins for the meningitis cultures. The *M. catarrhalis* agglutinated spontaneously, but the non-contact "W. n." took out all of its own agglutinins.

#### PATHOGENICITY.

Weichselbaum, in 1887, with his original cultures, killed white mice with an intraperitoneal or intrathoracic inoculation of 5 c.c. of a broth dilution of an agar culture or of the water of condensation. The mice died in 36 to 48 hours, and the meningococci were found in the cavity inoculated and usually in the blood. Subcutaneous inoculations were without result. He killed guinea-pigs by inoculating them in the thoracic cavity; but the cocci were not found in the blood or spleen.

Three dogs inoculated subdurally with 1 c.c. and 1.5 c.c. of culture dilution died, one the same evening, the second on the third day, and the third on the 12th day. The first two showed a small amount of fluid blood between the dura and brain. There was a small area of punctiform hemorrhages deeper in the brain, and the membranes were markedly injected. Numerous meningococci were found. In the third dog, between the dura and the right cerebral hemisphere, there was thick red pus, and in the brain a hazel-nut sized abscess containing yellow pus. Around the abscess was a hemorrhagic area. The lateral ventricles contained a red fluid with flakes of pus. No meningococci were found.

Albrecht and Ghon inoculated a goat in the spinal canal. The animal developed symptoms of meningitis and died in five days. The cord showed no changes and meningococci were not isolated.

Our animal work was rather irregular in its results. By inoculating mice intraperitoneally with half of a 24 hour ascitic agar culture of either the cord or nose strains, we caused death in 24 to 48 hours. There was marked congestion of the abdominal viscera, and meningococci were found in the blood and peritoneal exudate.

Rabbits were very uncertain. A few died from subdural inoculation of rather large doses, but there were no typical lesions, and none of them contained meningococci in the blood or exudate.

With small puppies we obtained about the same results as Weichselbaum. When given a dose of two ascitic agar cultures in the spinal canal, the dogs usually died in 24-48 hours. They had convulsions and some rigidity of the neck. On autopsy the membranes were much injected and there were hemorrhagic areas in the cortex. Meningococci were found in these areas, in the fluid under the dura, and in the spinal fluid. As controls to our meningococcus cultures, we used *M. catarrhalis* cultures and two cultures corresponding culturally to meningococci, which had been isolated from the nasal mucus of normal medical students. The dogs inoculated with two ascitic agar cultures of *M. catarrhalis* did not die, while those which received the cultures from the students died in 24 hours, and gave the same autopsy results as the dogs inoculated with meningococci.

#### CONCLUSIONS.

Meningococci were isolated from the nasal mucus of 50 per cent of meningitis patients during the first two weeks of the disease, and from about 10 per cent of the people most closely in contact with them. They were frequently present in enormous numbers.

The two cultures isolated from normal students were like meningococci culturally and in their pathogenicity, but did not have the same specific agglutinins.

The finding of meningococci in great numbers in the nasal mucus of such a large proportion of the patients and of those caring for them, and the absence of meningococci from the nasal mucus of a large number of normal persons examined, would strongly indicate the necessity of isolating cases of epidemic cerebro-spinal meningitis, at least during the early weeks of the disease.

We wish to thank Dr. Park for his constant oversight and direction of our work.

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## TEMPORARY ALTERATION OF CHARACTER OF AN ORGANISM BELONGING TO THE COLON GROUP.

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IN the spring of 1904 I was given the opportunity, through the kindness of my chief, Professor Adami, to study an organism isolated by him from the water of the St. Lawrence River. The results of these studies were published in the *Journal of Medical Research*,<sup>1</sup> and several interesting points in regard to the interagglutination of the Coli-Typhoid group were noted.

However, aside from the agglutination phenomenon, a peculiarity in the cultural characteristics was also observed. The microorganism on solid media resembled very much the appearance of colonies of *B. coli*. Grown on broth the microbe gave a stringy deposit, difficult to break up on shaking, and becoming more stringy on longer incubation. In litmus milk there was a primary acidity with a subsequent alkaline reaction of the medium, but no coagulation of the milk occurred. Indol was produced only after some weeks' incubation in Dunham's broth; and of the sugar broths, gas was produced most abundantly in the glucose medium. As was noted in my publication, the organism did not ferment lactose or saccharose when first isolated from the water, but did so after it had remained on the medium for some time. The saccharose broth was found to be more easily decomposed than the lactose, the latter medium showing only a very little gas formation after several days' incubation.

After the organism had been cultivated on artificial media for some months, the following experiment was reported:

A lethal dose of the bacillus was inoculated into the peritoneal cavity of a rabbit, and after its death (which resulted in three days), cultures were again obtained from it. The appearance of the organisms and the cultural characteristics were those of the bacilli inoculated, except that in the fermentation tubes there was a slight development of gas in the glucose broth, none in the lactose or saccharose. Transfers were made from these tubes into the respective sugar broths, that is, the glucose colony was transferred to glucose broth, the lactose colony to lactose broth, and the saccharose colony to the saccharose broth. After 24 hours' incubation there was an increased amount

<sup>1</sup> *Jour. Med. Res.*, 1904, 6, p. 475.

of fermentation in the glucose transfer, but still none in the lactose or saccharose. A second transfer was made, similar to the above, and now at the end of another 24 hours the glucose and saccharose broths were both fermented; no gas appeared, however, in the lactose. In both the glucose and saccharose there was also acid production. In the lactose broth no change was evident, though there was growth in the closed arm of the tube. Four days' incubation and transfer on lactose broth gave a small amount of gas formation, and transfers from this again into lactose led to its fermentation in 24 hours. The stock culture as control produced gas in all these sugar broths in 24 hours.

This feature of the organism, its variability in the power to break up certain sugars, presented two very interesting problems. First, are we justified in making an indefinite number of varieties of *B. coli*, depending on cultural characteristics which may be modified artificially; and secondly, in isolating from water an organism which in the first transfers does not ferment one or more definite sugars, but which, after remaining on artificial media for some time, acquires the property, can we conclude that the microorganism has recently had an animal host?

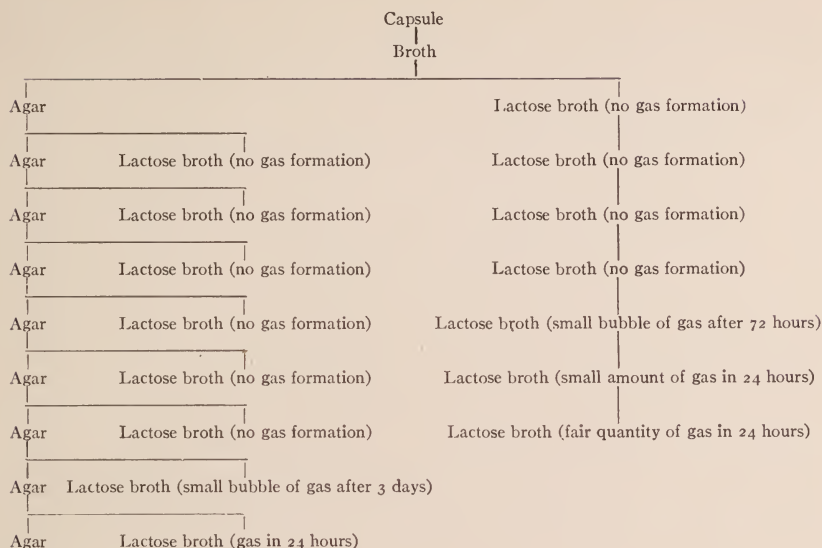
We have repeated our experiment of passing our microorganism, the *Bacillus perturbans*, through an animal. In this instance we made use of the celloidin capsules as devised by McCrae,<sup>1</sup> which we filled with a broth culture of the bacillus.

In the table it will be noted that two sets of transfers were carried forward, the one in which the parent culture was kept on sugar-free agar, and the other in which the parent stock was on a sugar medium similar to the transfer.

The sealed capsule was inserted, aseptically, into the abdominal cavity of a rabbit on September 19, 1904. The capsule was allowed to remain in the rabbit till February 10, 1905, in all 144 days. The capsule was then again obtained, and dropped for a moment without breaking into 10 per cent carbolic acid, after which it was placed in a flask of broth (without breaking), and incubated to insure against the chance of contamination. As no growth resulted, the capsule was ruptured and the microorganism was allowed to grow in weak broth for 18 hours. Transfers were then made into the various media, including sugar broths. The organisms resembled the original bacilli of the capsule in all the media except the sugars, though the growth was not in any case so luxuriant as transfers from the stock culture.

Transfers were made from and into the respective sugar broths daily. The glucose transfer showed a very slight gas formation on the first day, and this increased from day to day for several days. The first appearance of fermentation appeared in the saccharose on the fourth day, when it was only slight, and slowly increased with succeeding transfers. However, the lactose medium offered the greatest difficulty of fermentation, as can be readily appreciated from the following table.

<sup>1</sup>*Jour. Exp. Med.*, 1901, 5, p. 635.



As is seen in the above table, when the organism was transferred from a lactose to a lactose medium it regained its power to ferment lactose more rapidly than did the agar colonies. Having once acquired this property, the bacillus retained the lactose-splitting power in the successive transfers. In other words, the microorganism, having been deprived of one of its functions of altering the composition of certain materials by forced growth or environment, may again regain this function if it remain in contact with the material over an extended period.

Peckham<sup>1</sup> has given us the most complete study of the influence which environment exerts on the characters of organisms, especially of the colon group. In some cases this alteration consisted in an excessive activity of one function, in others the opposite, certain traits of the bacillus being entirely lost. In a series of cultural experiments, she was able to force *B. typhosus* to produce indol.

Of the external influences which can be brought to bear on bacteria, alteration of the quantity or quality of the food supply plays the most important rôle, and leads to modification of their biological nature. Thus some bacteria, in their normal metabolism, if we may so call the cell activity, secrete enzymes which split up proteids;

<sup>1</sup>*Jour. Exp. Med.*, 1897, 2, p. 540.

others secrete ferments acting on sugars. The colon bacillus, among others, possesses a proteolytic ferment, whose activity we estimate by the quantity of indol produced in the medium. If, however, the colon bacillus be grown over an extended time in river water, its power to produce indol is diminished or entirely lost; while again, as was said above, if a non-indol producing organism, such as the typhoid bacillus, be grown in a medium containing proteids alone, it acquires the property of producing indol.

Other examples of the influence of environment on bacteria are well known. Jenner<sup>1</sup> found that he could revert *B. coli capsulatus* to an unencapsulated form by cultural methods. The new variety then possessed characteristics dissimilar to the previous capsulated form; as for instance, while the capsulated bacillus coagulated milk, the unencapsulated stock lost this power when placed in this medium. A more remarkable difference was noted in the pathogenesis of these two varieties, for, as we know, *B. coli capsulatus* is very pathogenic for white mice, but becomes less fatal or even non-pathogenic on losing its capsule.

Experimenting with this same organism, *B. coli capsulatus*, Larulle<sup>2</sup> reports similar results of transforming his "opaque" variety into the "transparent," by passing the former through animals.

Other examples of alteration to a lesser degree in the characters of bacteria are seen in the everyday cultures, in the increase or decrease of the amount of acid produced, the morphological change which organisms undergo when inoculated on different media, and many other variations.

In a paper on the variability of bacteria Adami<sup>3</sup> discussed the alterations of character in bacteria due to environment, which give rise to different races of microorganisms. There he pointed out that two kinds of variations may occur, the temporary variation, in which the microorganism acquires characters that are lost after several transfers have been made, and the permanent variation, in which a new function or change is impressed on a microbe and remains with it in all future cultures. Of the latter class there are not many, for we must remember that what we call permanent is but a relative

<sup>1</sup>*Jour. Path. and Bact.*, 1898, p. 257.

<sup>2</sup>*La Cellule*, 1889, 5, p. 61.

<sup>3</sup>*Medical Chronicle*, 1892, 16, p. 366.



term. We speak of the characters as permanent when, after weeks, months, and years, no change is noted in the transfers from the type of the parent stock.

That at least temporary modifications can be brought about by such simple methods of cultivation and in so short a space of time seems to me to indicate that among those which we call varieties of *B. coli* there are some which owe their differentiating qualities to a prolonged habitat in a medium differing from that in which the parent stock has had its growth, and that through subsequent growth in suitable media the original qualities of the parent stock may be acquired. Our culture medium is at best a poor imitation of the natural habitat of these minute, and, I might say, impressionable, living bodies; hence we can conceive that investigators may obtain different results with the same organism. Thus with the colon bacillus it would seem that so long as we bring forward new sugars to ferment, we get an equal number of new varieties.

Further, when organisms, which under ordinary conditions produce gas in sugar media, are found to have lost this quality, it is one of the alternatives that the organism has been a parasite in the animal body. In our own case the same organism was also isolated from sewage flowing into the river, and the reactions of this strain of the microbe on media were the same as described, that is, it was primarily a non-lactose fermenter, but later acquired the property to break up this sugar.

## THE LONGEVITY OF BACILLUS TYPHOSUS IN NATURAL WATERS AND IN SEWAGE.

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### INTRODUCTION.

IN a paper published in this *Journal* in 1904,<sup>1</sup> Jordan, Russell, and Zeit detailed an extensive series of experiments on the longevity of the typhoid bacillus in water, in which simultaneous, though independent, tests were made on this organism as exposed to the waters of Lake Michigan, the Chicago River, the Chicago Drainage Canal, and the Illinois River. The methods used in this study and the results obtained were so different from those which have previously been reported that it seems desirable to test this question further, employing waters of different origin.

The attempt was made in all of this work to approximate, as closely as possible, the conditions that exist in nature, and, for this reason, a marked change in technique was instituted. Heretofore, it has been customary for experiments on the longevity of bacteria to be made in glass containers, filled with sterile or raw waters. The conclusions based on work under these conditions have been shown to be erroneous, and in the work previously referred to, the method was adopted of exposing the typhoid organism in permeable sacs (celloidin and vegetable parchment), filled with the type of water in which the sacs were suspended. If, then, any variation occurred in the composition of the stream in which the sacs were exposed, the influence of such variation, if of any effect, should be felt on the imprisoned cultures within the sac.

The results obtained in the experiments conducted on the Chicago Drainage Canal and other waters showed a marked variation in the vitality of *B. typhosus*. In the relatively pure waters of Lake Michigan, this organism could be recovered readily from the infected sacs, for a period of at least a week, while in the highly polluted waters of

<sup>1</sup> *Jour. Infect. Dis.*, 1904, 1, p. 641.

the Chicago River and the Drainage Canal, the longevity of the same strain, exposed in a similar way, was reduced to two days. These results were obtained with uniform regularity by all three observers, working independently, but employing the same general methods. The conclusions then drawn were of a tentative character, and it was deemed advisable to carry on further work. The studies here reported follow, in general, similar lines, using waters of different origin, under as widely diverse conditions as possible.

Special attention has also been given to the development of technical methods other than those previously used, so as to broaden, as far as possible, the basis upon which conclusions were to be made. Inasmuch as most of the technical methods used in the experiments here described are practically the same as those previously reported in the foregoing paper, it will not be necessary to repeat them in this connection. Only those modifications that further experience has demonstrated to be valuable, and the new methods that have been developed are here referred to.

These experiments have been made in the Wisconsin State Hygienic Laboratory at the University of Wisconsin. In part of the preliminary work much assistance was received from Mr. G. J. Marquette, then assistant in this laboratory. The waters used in these tests were from Lake Mendota, a spring-fed inland lake, of about 25 square miles extent, the waters of which may be regarded as fairly typical of those of a surface character. The sewage-infected waters were produced by adding to the lake water a given quantity of fresh liquid and solid human excreta.

#### METHOD OF EXPOSING THE TYPHOID BACILLUS.

In these experiments the exposure of the typhoid bacilli was made in the laboratory, rather than in the lake itself, the water, however, being piped for only a short distance. To place the infected sacs under conditions convenient for sampling and where they would not be subjected to the action of the weather, which was more or less troublesome in the Chicago series, the sacs containing the waters infected with the typhoid bacilli were placed in large tubulated glass receptacles, holding from two to three gallons, through which there was allowed to flow continuously a stream of water or sewage.

Reference to accompanying figure will indicate the arrangement of this device.

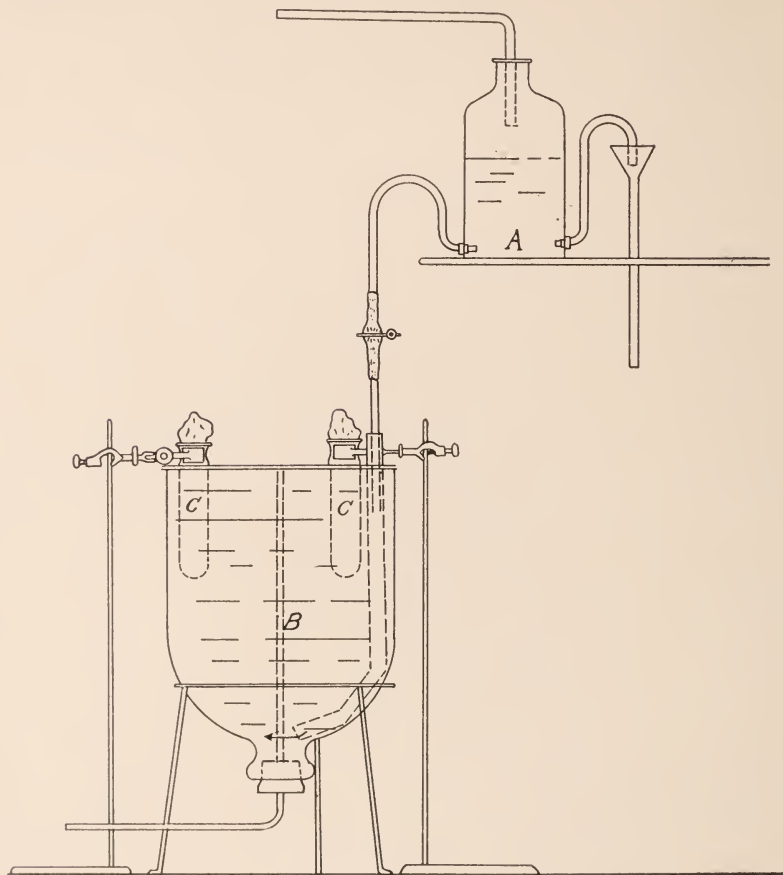


FIG. 1.

The sacs were immersed in the flowing stream so that the level of liquid in same was slightly below that of the water outside, thus maintaining a slight pressure toward the inside.

When exposed to the action of the lake water, the connection was made directly with the tap supplying this kind of water. To expose the sacs to the influence of sewage-infected waters, the device as shown in the cut was used. The sewage mixture was made up in a large reservoir placed in the attic, and from this was discharged through a pipe into a safety bottle, *A*, which regulated the flow into the reservoir below, *B*. The sacs, *C*, were held in position by clamping a rubber-faced clamp to a glass neck, which was sealed on to each kind of sac. The rate of flow was about six gallons per hour in the case of sewage, and a considerably higher rate for the lake water.



In the previous experiments, permeable sacs of celloidin and vegetable parchment were employed. In the work here recorded, another method has been devised, that of agar membranes. Some modifications of the previous methods have also been adopted.

1. *Celloidin sacs*.—The celloidin sacs employed have all been made by the extremely simple method of Frost,<sup>1</sup> in which the celloidin solution is poured on the inside of the test tube and the film, after it has been air-dried for the proper time, shrunk from the glass wall by means of water. By means of this method, sacs of practically any size can be made in a few minutes. These celloidin tubes were usually made to hold about 50 c.c. of water. They were held in position by inserting a glass neck of approximately the same bore as the sac, tying this on tightly with a soft-fibered thread, and coating the same with a layer of celloidin, allowing it to air dry. The sacs are filled with, as well as immersed in, water during the process of sterilization, which is done in an Arnold.

2. *Parchment sacs*.—In the former work sections of parchment tubing were used, such as is employed in dialysis work, but it is frequently difficult to secure tubing that is perfectly sound and free from minute holes. In this work, we have employed the parchment diffusion shells made by Schleicher and Schüll. These hold approximately 50 c.c. and are in the form of a tube closed at one end. Into the open, free end, a glass neck is fastened by means of sealing wax. The shells themselves are sterilized in streaming steam for an hour and a half, then allowed to dry under cover from the air. The glass necks can be sterilized chemically. The two parts can be quickly assembled in a sterile condition.

3. *Agar membrane sacs*.—The first introduction of agar for dialyzing purposes in bacteriological studies was made by Frost,<sup>2</sup> who used rectangular blocks about one-half inch square and an inch and a half long. These were made of plain agar and were inoculated by means of a stab in the center. The upper part of the stab was sealed off by dropping on melted agar, or smearing the upper surface of the block with a hot iron. Our first studies with agar were made with blocks of a similar character, but it was found that the

<sup>1</sup> Amer. Pub. Health Assoc. Rep. 1903, 28, p. 36.

<sup>2</sup> Jour. Infect. Dis., 1904, 1, p. 599.

layer of agar was too thick to permit rapid and complete dialysis. In some cases the typhoid organism, inoculated as a stab culture, died in a short time, while in other cases it persisted for a long period (several weeks).

The attempt was then made to use an agar film instead of the thicker block. It is necessary to have some mechanical framework to support the thin, delicate film of agar, and for this purpose cellulose diffusion shells, such as are used in chemical manipulations, have been found very serviceable. These are of the same size as the parchment shells previously referred to (38 by 85 mm.). After they have been sterilized in steam and allowed to dry, sterile glass necks of the same bore are inserted into the shells and fastened by means of sealing wax. The shell is then ready to receive its agar coating. Care is taken in the preparation of the agar to remove from it as much organic matter as possible. This is done by soaking the thread agar in distilled water for some hours, changing the water several times. A 2 per cent solution is then made. Tests made as to the nutritive properties of this agar showed that it would not support bacterial growth. Occasionally molds will make a sparse growth on the medium when left exposed to the air for some time. In coating the shells with the agar film, the material should be used in as hot a condition as it can be handled, so as to impregnate thoroughly the pores of the cellulose filter. It is advisable to pour some of the hot agar on the inside of the sac, rotating the sac quite rapidly, as in a roll culture, so as to distribute the material uniformly. The porous cellulose wall absorbs the liquid rapidly. After a little experience, one learns the requisite quantity of medium to employ in order to give a uniform and sufficient coating, and not have an excess. It is advisable to have the coating made at a single immersion, as the film is more homogeneous than where several applications are made.

When the inside coating has been properly applied, the sac is then dipped into the liquid agar and rotated so as to coat the outside of the sac also with a uniform layer. If the inside of the sac has not been coated in such a way as to exclude the air entangled in the cellulose meshwork, air vesicles will develop in the outer agar coat after a time and the integrity of the sac will be destroyed. Care

should be taken in this coating process to prevent infection of the sac, as it is of course impossible to sterilize the sacs after they are once made.

These agar films are not as durable as celloidin or parchment, but their integrity will be maintained unimpaired for about two weeks, or even longer, depending upon the nature of the liquid in which they are immersed. In water they retain their germ-tight properties longer than they do in sewage. In liquids very rich in bacteria, such as sewage, cytolytic enzymes are undoubtedly produced by certain types of organisms, thus softening the cellulose matrix and causing the sac to disintegrate.

While these agar membranes possess no point of superiority over the parchment or celloidin membranes formerly used, it is of importance to broaden the technical methods just as much as possible, and thereby determine if there are any essential variations in the results obtained which are due to the nature of the methods employed.

*Permeability of different types of sacs used.*—We have introduced into this study the use of a new type of permeable membrane, the agar film, and as no records have been reported on the question of relative permeability, so far as we know, it has been deemed advisable to incorporate here some of the results obtained in the study of these different types of sacs. The sacs used in this experiment are intended to hold in captivity the typhoid organism, and still at the same time subject this germ to the influence of diffusible substances that may be in the enveloping medium without.

In testing the permeability of these membranes, we have used simple, well-known substances that could be determined quantitatively, and have made no attempt to study the diffusibility of such materials as might possess an inhibitory effect on the imprisoned typhoid organism.

a) *Tests with chlorides.*—Experiments were first made with sodium chloride. Sacs were filled with distilled water and immersed in tap water, to which enough salt had been added to make the chlorine test 93 parts per million. Tests for chlorine were made on the contents of the immersed sacs at intervals of  $\frac{1}{2}$ , 1, 6, 12, and 24 hours. The results of these determinations are expressed in Table 1.

From the results herein shown it appears that a condition of nearly perfect equilibrium was established in all three sacs within a comparatively short time. The diffusion of the chlorides was somewhat more rapid in the agar and celloidin sacs than in the parchment, but within 24 hours' time, where no artificial currents were

TABLE 1.  
CHLORINE (PARTS PER MILLION) FOUND IN PERMEABLE SACS AFTER VARYING PERIOD OF IMMERSION  
IN SALT SOLUTION (93 PARTS PER 1,000,000).

	Agar	Parchment	Celloidin
$\frac{1}{2}$ hour.....	0	0	0
1 ".....	2.5	4	6
6 hours.....	63	50	76
12 ".....	80	67	84
24 ".....	84	76	86

used either within or without the sac to facilitate diffusion, from 81 to 92 per cent of the chlorine passed through the membranes of the sacs. In Fig. 2 are shown the data presented in the above table, expressed on a percentage basis of the total strength of the solution.

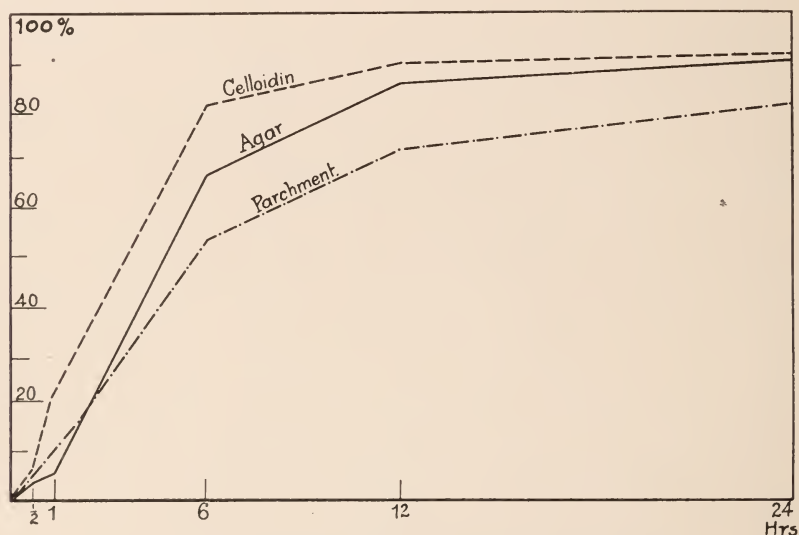


FIG. 2.—Relative permeability of different kinds of sacs to NaCl solutions

b) *Tests with sugars.*—In addition to the chlorides, tests were also made in the same way with sugar. Sacs filled with tap water were immersed in tap water containing 1.75 grms. of saccharose per 100 c.c. Quantitative determinations were made at  $\frac{1}{2}$ , 1, 6, 12, and 24 hour intervals with the following results:

TABLE 2.  
SUGAR (IN GRAMS PER 100 C.C.) FOUND IN PERMEABLE SACS AFTER VARYING PERIOD OF IMMERSION IN  
SOLUTION CONTAINING 1.75 GRAMS PER 100 C.C. WATER.

	Agar	Parchment	Celloidin
$\frac{1}{2}$ hour.....	0.03675	0.02625	0.20475
1 ".....	0.0630	0.0525	0.3850
6 hours.....	0.39475	0.22225	1.26525
12 ".....	0.6650	0.4270	1.4805
24 ".....	1.1375	1.0115	1.5925



These data, which are shown in graphical form in Fig. 3, seem to indicate that the celloidin type is much more permeable to sugar solutions than either agar or parchment. The rate of diffusion was not quite so rapid in this series as in the salt series, except in the case of the celloidin type.

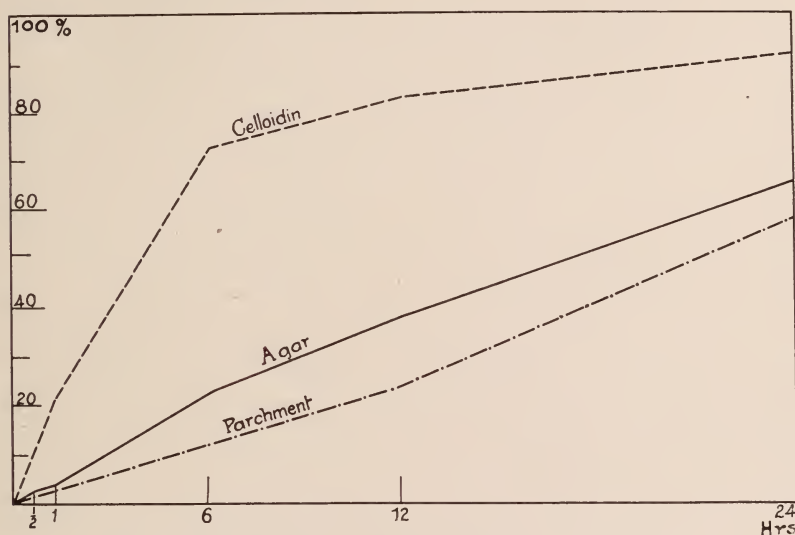


FIG. 3.—Relative permeability of different kinds of sacs to sugar solutions.

c) *Tests with peptone.*—Further tests were made with peptone solutions in order to test the permeability of the sacs to diffusible proteids. Sacs were filled with tap water and immersed in the same type of water, containing 1 per cent peptone. In these tests, a qualitative determination only was made. The contents of the sacs were tested for peptone by means of the biuret reaction at intervals of five minutes, until a positive reaction was obtained. The celloidin sac gave a positive reaction in 25 minutes, while the agar and the parchment required 35 minutes. The intensity of the reaction increased markedly in all cases after an hour's exposure.

It would appear from these tests, where various chemical substances of a widely different nature were employed, that the permeability of these different membranes was pronounced. On the whole, the results indicate that celloidin is the best membrane to employ, but this type is well supplemented by the addition of agar and parchment. Further corroborative evidence on the matter of permeability may also be presented in results that were noted in the actual prosecution of the work.

*Growth of bacteria in sacs immersed in nutrient solutions.*—If sacs of this character are sufficiently permeable to permit food substances in solution to pass the limiting membrane, it ought to be

possible to obtain growth of typhoid and water bacteria in sacs filled with water and immersed in liquids containing available organic matter.

To test this point more specifically, a special experiment was instituted. Six celloidin sacs were prepared and filled as follows: Two with sterilized tap water and the remaining four with raw tap water. The two sacs filled with sterilized water and two filled with raw tap water were inoculated with the same strength of typhoid suspension; the two remaining raw-water sacs were not infected with typhoid. These sacs were divided into two sets of three each. One set was immersed in running raw water, the other placed in sterile water containing 0.2 per cent peptone solution.

The results obtained in this experiment showed a most marked difference in the two sets of sacs, the peptone and the water series. In all three sacs of the peptone series a very marked growth was observed, both in the case of water bacteria originally present in the raw water, and the inoculated typhoid. The germ content of the control sac filled with raw water rose from a 32 colony count on plain agar on the first day, to 12,000,000 in the course of three days, and in 18 days had reached 40,000,000 bacteria per c.c. The sac filled with sterile water and infected with typhoid (16,000 per c.c.) underwent even a more pronounced growth than this. On the fourth day it contained 137,000,000 typhoid bacilli per c.c. From this high point the germ content gradually declined, but in 18 days there were still over 6,000,000 colonies per c.c., and the purity of the culture demonstrated the integrity of the sac. The course of changes followed by the sac containing the mixed flora (water bacteria + *B. typhosus*) underwent the same general change. On the 18th day 45,000,000 organisms per c.c. were demonstrable, and *B. typhosus* had been recovered in abundance on each intermediate day the test had been applied.

The course of changes noted in the water sacs was entirely different. The content of these sacs and the dosage was identical with the series immersed in the peptone solution. The only difference in this case was that the sacs were immersed in flowing tap water. The sac filled with raw water showed no growth on Drigalski-Conradi medium, and less than 50 bacteria per c.c. on plain agar.

Cultures were made for a period of six days, but no essential alteration in germ content was observed. The sac filled with sterile water and inoculated with *B. typhosus* (25,000 per c.c.) showed no increase. On the sixth day of the experiment it contained 17,000 organisms per c.c., apparently a pure culture (17 colonies picked all proved to be positive typhoids).

Accidentally the membrane was perforated on the seventh day, but for the period of observation reported no marked change had occurred in the germ content of the sac. The sac filled with raw water and infected with 30,000 *B. typhosus* fell to 3,000 on the third day. On the fifth day *B. typhosus* was recovered, but on the sixth day none could be found in culture plates containing about 100 colonies per c.c.

The results of this series are wholly consistent, and show that both water bacteria and *B. typhosus* are capable of multiplying extensively in raw waters, as well as sterile, where such waters are exposed in permeable sacs in liquids containing available food material in solution.

This experiment conclusively demonstrates the permeability of the sacs to such solutions, and would seem to show beyond all reasonable doubt that if bacteria die rapidly when imprisoned in such permeable cages, they do not succumb because of the inability of food substances in outside enveloping liquid to pass the limiting membranes of these sacs.

In addition to this carefully controlled experiment with peptone solutions, observations were made in the course of the experiments later detailed, which also throw light on this point. In series VIII a sac filled with raw water, but inoculated with typhoid, was immersed in a bath of flowing sewage. In Table 16 is shown the bacterial content of this sac on various days. A phenomenal development of the water bacteria occurred in this case, as in the peptone solution, showing that sewage also contains sufficient good material, which was able to permeate the celloidin membrane to give the water bacteria in raw water a favorable environment for rapid growth.

These results, taken in connection with the specific tests made as to permeability, would seem to indicate that the methods employed

permitted diffusion to occur with sufficient rapidity so that the conditions approximated those that prevail in a flowing stream.

*Germ-proof qualities of sacs.*—In using sacs of a permeable character, it is of the utmost importance that they should be relatively germ tight. The celloidin sac has been tested for so long a period of time that there is no longer any question as to the tightness of sacs of this type, but concerning the use of parchment and agar, this question may well be raised. In our earlier experiments, the attempt was made to rely on parchment tubing, such as is used in dialysis work, but we have been much troubled to get satisfactory tubing of this character. The process of making the cellulose fiber into vegetable parchment seems to destroy the pliability of the material, so that it cracks more readily upon bending. In this way minute breaks or punctures are often to be noted. Since the adoption of the parchment diffusion shells, no trouble of this character has occurred.

The agar sacs are, of course, relatively fragile so far as the film is concerned, and the filter-paper matrix on which the agar is spread is not as permanent as parchment. When immersed in liquids rich in bacterial life, such as sewage, the cytolytic enzymes cause the disintegration of the cellulose fibers, this occurring more rapidly in the filter paper than with the parchment sac. In purer types of waters this rotting does not occur so readily. We have rarely had any trouble with sacs of this character, if the experiment did not exceed two weeks' time.

When the sacs are allowed to remain in dilute sewage, or even in water after a considerable lapse of time (10 days or more), a somewhat slimy growth is formed, as is also the case on the inner face of the glass receptacle. This is easily removed by brushing the sacs occasionally with a camel's hair brush.

Experiments were made with the special object of testing the integrity of these types of sacs. Sterile sacs were filled with sterilized water and immersed in either water or sewage. The result of such tests, even where continued for a week or more, showed no passage of bacteria through the sac membrane.<sup>1</sup>

<sup>1</sup> Johnson (*Eng. Rec.*, Sept. 23, 1905, also *Jour. New Eng. Water Works Assoc.*, 1905, 10, p. 508) has questioned the integrity of sacs of this character, but the experimental data given by this writer are too meager to permit of any weight being attached to his conclusions.



In some of the actual tests performed in these studies (see Series VII and VIII), it was possible to throw light on the matter of tightness of the sacs. Sacs filled with lake water and inoculated with typhoid were immersed in sewage. Drigalski-Conradi plates were made at daily intervals. If any leakage had occurred, it would have immediately manifested itself by the appearance of acid colonies on this medium, as, of course, the colon type was abundant in the dilute sewage outside of the sac. In most of the sacs no evidence of any leakage occurred. In one or two of them slight evidence of leakage was discovered in the course of 13 to 14 days. We are, however, of the opinion that any one of these three methods may be relied upon to maintain readily a germ-tight, and yet permeable, membrane, if any degree of care is used in selection and manipulation of the sacs.

#### CULTURES EMPLOYED.

In order that this work might be directly compared with that of the previous year, one of the same typhoid strains that was employed in the Chicago Drainage Canal work has been used throughout all of these experiments. This culture, strain "Y," was isolated October 1, 1903, under Professor Jordan's direction, from the urine of a typhoid patient. The case was typical in its symptoms, and the blood of the patient gave a positive agglutination test on the tenth day.

#### METHOD OF RECOVERY OF TYPHOID BACILLUS FROM SACS.

Where the typhoid organism is in direct contact with water or sewage forms, it is advisable to employ some of the special methods that have been devised for the differential cultivation of this organism. For this purpose we have employed throughout this work, the Drigalski-Conradi medium,<sup>1</sup> modified somewhat by the omission of nutrose. By filtering the mixture before adding the litmus solution, the flocculent precipitate is much reduced.

The use of a culture medium like the D.-C. medium is of great value in inhibiting bacterial growth which would otherwise obscure the typhoid organism. While the addition of the crystal violet does not completely inhibit water bacteria, it reduces materially the germ content, as is shown from the following data, where cultures were made on plain nutrient agar and on the D.-C. medium.

<sup>1</sup> *Ztschr. f. Hyg.*, 1902, 39, p. 282.

TABLE 3.  
RELATIVE GERM CONTENT OF WATER SACS ON AGAR AND ON DRIGALSKI-CONRADT MEDIUM.

Date	Amount Used	Medium Employed	Sac 1	Sac 2	Sac 3	Sac 4
1904						
December 15.....	1 c.c.	Plain agar	6,500	3,900	730	1,620
	1	D.-C. medium	750	410	150	350
December 16.....	1	Plain agar	6,500	1,050	1,530	1,150
	1	D.-C. medium	560	300	137	175
December 17.....	1	Plain agar	5,900	4,100	1,450	6,500
	1	D.-C. medium	1,150	4,300	490	150

#### RAPID IDENTIFICATION OF TYPHOID ORGANISM.

In determining whether any organism isolated from the plate cultures is true typhoid or not, it has generally been customary to test the presumptive organisms by first passing them through dextrose agar shake cultures, or stabs. From this, if no gas was produced, transfers were made into litmus milk and gelatin, and tests for indol were made. To differentiate it from *B. alkaligenes*, which develops no acid in litmus dextrose broth, cultures were made in this medium. If the organism in question stood all these culture tests satisfactorily for typhoid, it was then finally tested for agglutination with a highly potent typhoid-immune serum. Such a procedure as this involves a large number of transfers. In the course of these studies, a modification of the above method was made, which is as follows:

The Drigalski-Conradi plate cultures were carefully studied to pick out the presumptive typhoid colonies. This was not always easy to do, as there are many organisms occurring in sewage, or even in water, that are capable of development in this crystal violet medium, and which retain the blue color. Many of these can, of course, be easily rejected, as they are too luxuriant in their growth, being thick and opaque. But, not infrequently, types of colonies of a thin, semi-transparent blue cast have appeared, that more or less closely resembled the true typhoid. In picking out the presumptive typhoids, it is advisable to have on hand, for purposes of comparison, several culture plates made from a pure typhoid strain.

A more rapid method of identification was devised as follows: The presumptive typhoid-like colonies were fished and subcultured directly into litmus *dextrose* agar, by making a combination streak and stab culture. In this medium the typhoid, of course, formed acid, but no gas, and was thus easily differentiated from Petruschky's *B. fecalis alkaligenes*, which remained blue, not only in lactose but in glucose litmus media. Organisms of the colon type would naturally acidify dextrose agar, but these are excluded on the D.-C. culture plates. If, perchance, colon types should be transferred, their presence would be manifest by the copious gas production.

Following the litmus glucose test, all non-gas-producing acid forms were then subjected directly to the macroscopic agglutination test with typhoid immune sera. If the isolated cultures stood these two tests, they were considered positive typhoids. Upon the completion of these tests, a number of cultures from each series were selected at random and subcultured on all the usual media, as gelatin, milk, and

glucose-free broth for indol, so as to check still further the culture characteristics of the supposed typhoid cultures.

Our experience with this short method of identification leads us to recommend its use over the longer method. We have found no organism in normal waters that is liable to be confused with the typhoid, where reliance is placed on these tests. In sewage, however, there is a blue type of organism that appears in 24 to 48 hours on Drigalski-Conradi plates, and which, therefore, might be transferred to the litmus dextrose cultures, on which it forms acid. This type has invariably failed to be agglutinated with typhoid-immune sera. Later, if one studies the original plate cultures, after a more prolonged period of incubation, he finds that these colonies are faintly acid. Evidently they are able to produce acid very slowly on lactose media.

#### OUTLINE OF EXPERIMENTS MADE.

In order to present a general summary of the work done in this series of studies, a synoptical table is presented below, in which the varying conditions, as to manner of exposure, dosage, temperature, etc., are shown.

TABLE 4.  
SYNOPSIS OF DIFFERENT SERIES OF EXPERIMENTS MADE.

	No. of Series	No. Sacs Used	Kind of Sacs	Approximate Typhoid Dosage per c.c.	Nature of Liquid in Sac	Nature of Liquid in Outside Container	Range in Temperature (° C.)
LAKE WATER	I	2 2	Agar Celloidin	110,000-200,000 110,000-200,000	Lake water " "	Lake water " "	10-14 10-14
	II	1 1	Agar Celloidin	25,000 85,000	" " " "	" " " "	9-12 9-12
	III	1 2	Celloidin Parchment	150,000 120,000-150,000	" " " "	" " " "	15-18 15-18
	IV	1 1	Celloidin Glass	2,275,000 1,709,000	" " " "	" " .....	21-23 21-23
SEWAGE	V	1 2 1	Parchment Celloidin Agar	1,500,000 5,000,000 10,000,000	Sewage " "	Sewage " "	21-29 21-29 21-29
	VI	1 1	Parchment Celloidin	4,350,000 5,800,000	" "	" "	22-25 22-25
SEWAGE IN WATER	VII	2 2 2	Celloidin Parchment Agar	190,000-300,000 " " " "	Lake water " " " "	Sewage " "	15-19 15-19 15-19
	VIII	2 1	Celloidin Parchment	2,275,000 1,706,000	" " " "	" "	22-25 22-25
	IX	1 1	Celloidin Parchment	100,000 200,000	" " " "	" "	22-25 22-25
SEW'GR IN WATER	X	1	Parchment	4,350,000	Sewage	Lake water	21-23

## PART I.

## BACILLUS TYPHOSUS EXPOSED TO LAKE MENDOTA WATER.

*Series I. Agar and celloidin sacs filled with lake water and immersed in lake water.*—This series was started on December 5, 1904. Two agar sacs and two celloidin sacs were filled with raw lake water, and each sac inoculated with two large loopfuls of a 24 hour culture of the "Y" strain of the typhoid bacillus. These cultures were exposed continuously to the action of flowing lake water from the above date until December 22, making a period of 17 days in all.

The temperature of the water as it reached the exposed cultures was about 12° C. Quantitative plate cultures were made on the D.-C. medium throughout the whole experiment, in order to note the relative rate of decline of bacteria within the sacs. In the beginning three plates were made daily from each sac, using  $\frac{1}{10}$ ,  $\frac{1}{2}$ , and 1 c.c. of the infected water, respectively. From this set of plates a very fair average could be determined, and the accuracy of the work thus checked by the quantitative findings with different dilutions. As the work progressed and the colony count per c.c. fell from the high initial number to lower limits, the quantity used in each seeding was materially increased. Under such conditions it is highly improbable that typhoid organisms were overlooked, where all typhoid-like colonies were removed and tested.

The quantitative findings of this series are presented in Table 5, from which it is apparent that the high initial content (which was, of course, largely composed of typhoid organisms at the beginning) underwent a rapid and continuous decline from the beginning to the seventh day. By this time the average number of organisms in the four different sacs had fallen from the initial seeding of 147,700 per c.c. to 2,170 per c.c. From this period to the end of the experiment, the number of organisms per c.c. remained very small, ranging at most from a thousand or so to a few hundred.

TABLE 5.  
NUMBER BACTERIA PER C.C. IN TYPHOID-INFECTED SACS AFTER EXPOSURE TO FLOWING LAKE WATER.

Date of Test after Infection	Temperature	Sac 1 Agar	Sac 2 Agar	Sac 3 Celloidin	Sac 4 Celloidin
4 hours.....	12° C.	130,000	111,800	203,000	146,000
2d day.....	12	34,800	43,000	152,000	99,250
3d ".....	12	41,000	45,500	100,000	90,000
4th ".....	14	20,500	33,000	58,500	62,500
5th ".....	14	16,000	28,600	49,000	46,250
6th ".....	14	13,750	22,250	30,750	33,250
7th ".....	7	2,350	900	2,405	3,315
8th ".....	9	1,260	500	1,520	1,180
9th ".....	10	1,720	360	280	420
10th ".....	12	750	410	150	350
11th ".....	13	500	300	135	175
12th ".....	12	1,150	500	400	150

This comparative decline is also shown graphically in Fig. 4.

The marked diminution in numbers noted in the above table is unquestionably due to the rapid destruction of the typhoid bacilli inoculated into the raw water with which the sac was filled. The initial germ content of this water before inoculation with typhoid ranged from 500 to 1,000 bacteria per c.c., but unquestionably some increase in water bacteria would occur even in these permeable sacs. As the D.-C. medium was used throughout all these tests, these water bacteria would be largely prevented



from development, so that the apparent diminution doubtless registers the decline in typhoid content.

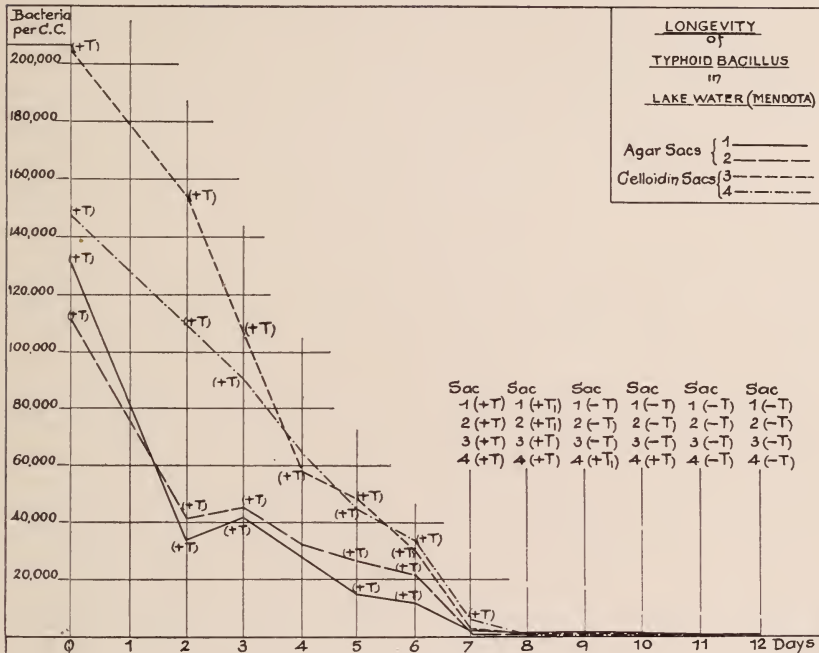


FIG. 4.

To check these quantitative results, extensive tests were also made to determine the longevity of the inoculated typhoid organisms by removing pure cultures from the Drigalski plates each day, and subculturing them.

The results of these qualitative tests are presented in Table 6, in which are given the number of organisms fished from the plates and also the number proven to be typhoid upon the basis of the agglutination test and subcultures. There was no difficulty in recognizing the typhoid organism in the plates during the earlier part of the series, as they were crowded with colonies of a similar character, and it was unnecessary to fish a large number. As the inoculated typhoid organism gradually died out, the colony appearance on the plates became more diverse, and the number of presumptive typhoid colonies was greatly diminished.

The results obtained in this series of tests are certainly very striking. A large proportion of the genuine typhoid colonies were found among those fished on the earlier days of the series. In the case of the agar sacs, as late as the seventh day, a majority of all fished colonies proved to be typhoid, while in the celloidin sac, this persistence was very marked, until a day later (eighth day). After

TABLE 6.

LONGEVITY OF *B. TYPHOSUS* IN AGAR AND CELLOIDIN SACS IN LAKE MENDOTA WATER

	SAC 1 (AGAR)		SAC 2 (AGAR)		SAC 3 (CELLOIDIN)		SAC 4 (CELLOIDIN)		TOTALS	
	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found
4 hours.....	3	3	4	4	2	2	4	4	13	13
2d day.....	8	7	9	9	9	8	9	5	35	29
3d ".....	10	9	9	8	8	8	5	4	32	29
4th ".....	11	4	10	4	11	9	6	6	38	23
5th ".....	5	1	10	8	8	8	9	8	32	25
6th ".....	9	5	10	8	10	9	8	7	37	20
7th ".....	10	4	8	5	7	7	12	8	37	24
8th ".....	3	1	4	1	4	3	6	6	17	11
9th ".....	2	..	2	..	2	..	2	1	8	1
10th ".....	1	..	1	..	5	..	5	1	12	1
11th ".....	2	..	..	..	..	..	..	..	2	..
12th ".....	6	..	..	..	3	..	7	..	16	..
13th ".....	1	..	2	..	..	..	..	..	3	..
17th ".....	..	..	..	..	6	..	..	..	6	..
	71	34	69	47	75	54	73	50	288	186

these dates a very pronounced diminution in typhoid colonies appears. Scattering colonies were found in the agar sacs on the eighth day, but none later, and in one of the celloidin sacs a similar condition was observed on the 9th and 10th days, but none could be found subsequently. The appearance of the culture plates in the earlier and later periods of this series showed a marked difference in colony aspect. In the first week the plates were studded with apparently typically typhoid colonies, which upon subculture proved to be genuine typhoid by the different culture tests and the agglutination reaction. After this period (8 or 10 days), the aspect of the colonies appearing on the culture medium was of an entirely different character, and only rarely did any forms appear that could be suspected of typhoid relationships. In no case, however, did any of these organisms prove to be typhoid.

*Series II. Agar and celloidin sacs filled with lake water and immersed in lake water.*—This series, which was run prior to that just described above, is not as satisfactory, in that the data acquired are not nearly as complete as they should be, but it is presented as furnishing evidence of the action of lake waters under winter conditions. The series consists of two sacs, one agar and one celloidin, both of which were filled with raw lake water and inoculated with a typhoid dosage of 25,000 and 85,000 typhoid bacilli per c.c., respectively. The sacs were then immersed, as before, in flowing lake water, having a range in temperature from 9° to 12° C. The data as to the presence of the inoculated typhoid bacilli are presented in Table 7.

TABLE 7.

LONGEVITY OF B. TYPHOSUS IN AGAR AND CELLOIDIN SACS IN LAKE MENDOTA WATER.

	TEMP. ° C.	SAC 5 (AGAR)		SAC 6 (CELLOIDIN)		TOTALS	
		No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found
3 days.....	14	7	4	10	2	17	6
5 ".....	12	6	0	2	0	8	0
8 ".....	12	11	4	9	1	20	5
12 ".....	14	4	0	5	0	9	0
		28	8	26	3	54	11

While the number of colonies removed in this case was not large, yet the same general result was obtained as before. It was readily observable on the plates made the third day after seeding that the number of typhoid colonies had undergone a marked reduction. While a daily study of this series was not made, yet the results, as far as they go, confirm in general the conclusion of the preceding series. A marked reduction in total colony count was observable after the third day. The last time typhoid was found was on the eighth day.

TABLE 8.

LONGEVITY OF B. TYPHOSUS IN PARCHMENT AND CELLOIDIN SACS IN LAKE MENDOTA WATER.

	SAC No. 7 (PARCH.)		SAC No. 8 (PARCH.)		SAC 9 (CELL.)		TOTALS—ALL SACS	
	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found
1 hour.....	4	4	4	3	4	3	12	10
1 day.....	6	0	6	2	4	4	16	6
2 days.....	5	1	8	1	4	2	17	4
3 ".....	8	2	8	0	5	5	21	7
4 ".....	5	2	8	1	7	0	20	3
6 ".....	6	2	10	4	7	1	23	7
8 ".....	10	1	6	0	9	0	25	1
10 ".....	4	0	3	0	3	0	10	0
11 ".....	4	0	3	0	3	0	10	0
13 ".....	4	0	3	0	1	0	8	0
15 ".....	1	0	..	..	1	0	2	0
17 ".....	1	0	2	0	..	..	3	0
	58	12	61	11	48	15	167	38

*Series III. Parchment and celloidin sacs filled with lake water and immersed in lake water.*—Another series in lake water was begun on May 2, 1905, in which the same general arrangement as before was followed. One celloidin and two parchment sacs were immersed in lake water, after being filled with lake water, which at this time contained 140 bacteria per c.c. The seeding of typhoid in this case ranged from 120,000 to 150,000 bacteria per c.c. Determinations made of the germ content of the lake water during the progress of the experiment, which lasted from May 2 to May 17,

showed at all times less than 400 bacteria per c.c. The temperature under these summer conditions ranged from 15° to 18° C., and was materially higher than in the two preceding series. The results of this test are shown in Table 8.

Here again there is practical unanimity as to the results obtained in the parchment and celloidin sacs. Typhoid colonies were detected in all three sacs on the sixth day and in one sac as late as the eighth day, although only one colony was found. Cultures were continued for a period of 17 days, but no typhoid colonies were recovered after the period mentioned. In this series the typhoid type could be differentiated on the culture plates with a greater degree of accuracy than in the preceding Series I. An attempt was made to estimate, only approximately, of course, the number of typhoid colonies that developed on the various plates. These data cannot be relied upon implicitly, because one cannot be absolutely sure as to whether a colony is typhoid or not, where reliance is placed on the culture-plate appearance. But the plates were held in the ice box until after the first picking had been run through the necessary differential tests, so that the differentiation was more accurate than it otherwise would have been. The following records give the approximate number of typhoid colonies on the various plates for the different days of exposure.

Date	Days of Exposure	Character of Culture Plates
May 2.....	0	Typhoid type greatly predominating
May 3.....	1	Total colonies much diminished, about 8,000 typhoids per c.c.
May 4.....	2	About 700 typhoids on 1 plate; less on other 2 cultures
May 5.....	3	1 plate (1½ c.c.) had clump of about 50 typhoids, besides other scattering colonies. Both other plates contained a few
May 6.....	4	Several score typhoids on plate 1, 20-30 each on plates 2 and 3
May 8.....	6	40 typhoids on parchment sac from which 4 fished colonies were proven to be genuine typhoid. Only sporadic colonies on plates of other sacs
May 10.....	8	Character of plates completely changed. No typhoid observed except the single colony isolated from parchment sac
May 12.....	10	¼ and ½ c.c. water now used in culture plates. Germ content 300-500 colonies per plate, but all negative typhoid
May 15.....	13	1 c.c. cultures. Germ content trivial on D.-C. plates
May 19.....	17	1 c.c. cultures. D.-C. plates nearly sterile. Lactose agar about 150-200 colonies per c.c.

Thus, from an ocular inspection of the culture plates, as well as from the completed study of the isolated cultures, death is to be noted, in the course of a few days, of the hundreds of thousands of typhoid bacilli introduced into the lake water at the beginning. The great majority of these organisms disappeared in the course of a



few days (three or four), and after the lapse of six days, they could only be found in sporadic cases.

*Series IV. Celloidin sac and glass tube filled with lake water, and immersed in lake water.*—This series, run from August 15–24, 1905, included a celloidin sac and a glass tube of similar size. The dosage of the two containers was quite heavy, 2,275,000 and 1,706,000 typhoid bacilli per c.c. respectively. The temperature ranged from 21° to 23° C. during the work.

TABLE 9.

LONGEVITY OF *B. TYPHOSUS* IN CELLOIDIN SAC AND GLASS CONTAINER IN LAKE MENDOTA WATER.

	SAC 10 (CELLOIDIN)		SAC 11 (GLASS CONTAINER)		TOTALS	
	No. Col. Fished	No. Proven Typhoid	No. Col. Fished	No. Proven Typhoid	No. Col. Fished	No. Proven Typhoid
1 hour.....	17	11	10	10	27	21
2 days.....	15	5	4	4	19	9
4 ".....	29	10	7	3	36	22
6 ".....	8	0	17	0	25	0
7 ".....	12	0	18	6	30	6
8 ".....	25	2	14	1	39	3
10 ".....	7	2	30	6	37	8
13 ".....	6	0	18	1	24	1
14 ".....	4	0	22	0	26	0
	123	39	140	31	263	70

In this series in the permeable sac, the inoculated typhoid was found as late as the 10th day, while in the glass container, immersed in the reservoir so as to maintain exactly the same temperature, it persisted until the 13th day. This result is in accord with data previously collected, where experiments have been carried on in glass receptacles, in which case it is generally found that the longevity of the typhoid organism is materially prolonged. For this reason, the earlier work on this question of longevity cannot be regarded as conforming to conditions that obtain in nature.

#### SYNOPSIS OF EXPERIMENTS WITH TYPHOID BACILLI EXPOSED TO NORMAL LAKE WATER.

In the foregoing series (I–IV) there is, on the whole, a striking agreement as to the length of time that the typhoid organism could be detected. In two respects considerable variation was to be observed in these series, viz., temperature of exposure and dosage, but the longevity of the introduced organism ranged through a comparatively narrow period of time (8–10 days). Two of these series were carried out in the winter, one in the spring, and another in the summer, when the average temperature of the water ranged

from 21° to 23° C. In Series III, which was made in May, the organism persisted for the minimum period of time, and it was thought possible that this might be ascribed to the higher temperature; but in Series IV, where the temperature was still higher, *B. typhosus* persisted for the usual period (8-10 days). In this case, though, the typhoid dosage was high, as the sacs were inoculated with approximately 2,000,000 organisms per c.c., a number much larger than usual.

In Table 10 is presented a summary of the results obtained in all these series, and, for purposes of comparison, the data collected last year by Professor Zeit in the work on Lake Michigan are also incorporated.

TABLE 10.  
SUMMARY OF EXPERIMENTS ON LONGEVITY OF *B. TYPHOSUS* EXPOSED TO SURFACE WATERS (LAKE MENDOTA AND LAKE MICHIGAN).

SERIES	KIND OF SAC	SAC No.	DAYS																	TOTAL No. COL. FISHED	No. PRO- VEN TY- PHOID COL.
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
I	Agar.....	1	+		+	+	+	+	+	+	+	0	0	0	0	0			0		
	".....	2	+		+	+	+	+	+	+	+	0	0	0	0	0			0		
	Celloidin.....	3	+		+	+	+	+	+	+	+	0	0	0	0	0			0		
	".....	4	+		+	+	+	+	+	+	+	+1	+1	0	0	0			0		
II	Agar.....	5							+		+				0	0					
	Celloidin.....	6							0		+1				0	0					
III	Parchment.....	7	+	0	+	+	+		+		+1			0	0	0		0	0		
	".....	8	+	+	+	0	+		+					0	0	0		0	0		
VI	Celloidin.....	9	+	+	+	+	0		+1		0			0	0	0		0	0		
	Celloidin.....	10	+	+	+	+	0		0		0			0	0	0		0	0		
	Glass.....	11	+		+		+		0	+	+		+			+	0				
																				288	186
																				54	11
																				167	38
																				213	63

LAKE MICHIGAN (ZEIT).																					
Parchment.....	..	+	+	+		+		+	+	0		0	0								
".....	..	+	+	+	+	+		+	+	0		0	0		0						
".....	..	+	+	+	+	+		+	+	0		0	0		0						
".....	..	+	+	+	+	+		+	+	0		0	0		0						
Celloidin.....	..	+	+	+	+	+		+	+	0		0	0		0						

+ means that typhoid was found more or less abundantly; 0 indicates complete disappearance of typhoid; +1, where figure "1" follows the plus sign, signifies that the positive typhoid findings were reduced to a single colony.

Comparing these two types of surface waters, one from an inland lake of moderate dimensions, the other from a very much larger water reservoir, it appears that the results of these two sets of experiments are not greatly different. In Professor Zeit's work, the average period of longevity was about seven days, while in our studies it has ranged from 8 to 10 days. Still, by far the most of the typhoid

organisms disappear before the end of a week. It is, however, necessary to set the limit at complete disappearance, although it has been generally noticed that there are often a few seemingly more resistant individual germs that persist for an appreciably longer time than the average.

## PART II.

## BACILLUS TYPHOSUS EXPOSED TO DIRECT INFLUENCE OF FRESH SEWAGE.

Having determined the relation of the typhoid organism to such natural surface waters as those of Lake Mendota, in which the normal bacterial content is relatively low, we next directed our attention to the question of the longevity of this organism when exposed to the influence of liquids rich in germ life and their products of growth. The previous work<sup>1</sup> on the waters of the Chicago Drainage Canal had indicated that the typhoid bacillus was unable to survive in highly polluted waters for as long a period of time as when exposed to a purer type of surface waters. This conclusion was made in a tentative way, but the importance of it in sanitary work is such that further study is desirable.

The purpose of the following series was to repeat this work on sewage in order to test the validity of the tentative conclusion previously drawn. For this purpose sacs were filled with fresh sewage, inoculated heavily with the same strain of the typhoid bacillus previously used, then immersed in a reservoir through which a stream of fresh sewage was allowed to flow.

*Series V. Parchment, celloidin, and agar sacs filled with sewage and immersed in flowing fresh sewage.*—Under the conditions of this series, the typhoid bacillus was exposed to the direct influence of the sewage organisms themselves, as well as their by-products of growth. In carrying out these experiments, it was necessary to use a much wider range of dilutions in making the cultures, in order to give the introduced bacillus most favorable opportunities for development. The typhoid dosage used was naturally much larger than that employed in the preceding cases. As was customary with the plate cultures made in the previous series, all plates were saved after they had been subjected to the usual examination, and all presumptive typhoid colonies marked and subcultured. The plates were then allowed to develop further, and after the discontinuance of sampling, the entire series, as a whole, was subjected to a comparative study, and the second crop of typhoid-like colonies removed. By subjecting the plate cultures to this comparative study, it is believed that it was possible to locate all typhoid organisms that developed on the plates.

In this series, three sacs (one each of celloidin, agar, and parchment), were filled with sewage and then immersed in flowing sewage. These sacs received respectively 1,500,000, 5,000,000, and 10,000,000 bacteria from a 24 hour culture of the "Y" strain.

Another celloidin sac, filled with sewage of the same composition, but not inoculated with typhoid, served as a control to study the course of the bacterial changes in the sewage itself.

<sup>1</sup> *Jour. Infec. Dis.*, 1904, 1, p. 641.



The sewage solution was made by mixing fresh human excreta (liquid as well as solid) with lake water and holding the mixture in a reservoir containing about 35 gallons. This reservoir was filled from time to time to maintain a continuous flow. As judged by appearance and odor, the sewage was fairly strong. Chlorine determinations were made at intervals, but naturally there was considerable fluctuation, depending upon the introduction of the urine. At the beginning of the experiment the sewage contained 244 parts of chlorine per million, while at the end there were 340 parts. The chlorine content was, however, lower than this during the progress of the experiment.

The following observations were made on the germ content of the sewage in the outside reservoir and within the control non-typhoid-infected sac.

TABLE II.  
BACTERIAL CONTENT PER C.C. OF SEWAGE.

	DAYS OF EXPOSURE	OUTSIDE FLOWING STREAM		INSIDE OF CONTROL SAC
		Lactose Agar	Drigalski-Conradi Medium	Lactose Agar
July 13.....	0	7,875,000	3,500,000	10,500,000
July 15.....	2		250,000	6,350,000
July 17.....	4		5,185,000	5,150,000
July 26.....	13		7,100,000	

In all cases an abundance of acid colonies on the D.-C. medium indicated the presence of sewage types. The temperature of the flowing liquid ranged from 21° to 29° C., with an average for the whole period of 24.8° C.

*Results obtained in series V.*—The examinations made on this series were begun on July 13 and continued on most of the sacs til July 27, covering a period of 14 days. At this time the character of the culture plates indicated that the typhoid type had entirely disappeared, and from previous experience it was deemed inadvisable to continue sampling longer. The results of this series are briefly summarized in Table 12, in which are given, for the respective days, (1) the total number of colonies picked, (2) the number which were regarded as presumptive typhoids on the basis of the litmus glucose agar test, and (3) the number of proven or verified typhoid colonies as determined by the agglutination and the differential culture tests.

The results obtained in all three sacs, including parchment, agar, and celloidin types, are in striking agreement with each other. In the three different kinds of permeable sacs employed, the results were identical. The last typhoid organism was found in each sac on the fifth day of exposure, but it is noteworthy that a marked decline

TABLE 12.  
LONGEVITY OF *B. TYPHOSUS* EXPOSED IN PERMEABLE SACS FILLED WITH SEWAGE AND IMMERSSED IN FLOWING SEWAGE.

DAYS	SAC 12 (AGAR)			SAC 13 (PARCHMENT)			SAC 14 (CELL)			TOTALS DIFF. DAYS		
	No. Col. Fished	No. Pres. Typh. Found	No. Prov'n Ty-phoid	No. Col. Fished	No. Pres. Typh. Found	No. Prov'n Ty-phoid	No. Col. Fished	No. Pres. Typh.	No. Prov'n Ty-phoid	No. Col. Fished	No. Pres. Typh.	No. Prov'n Ty-phoid
0.....	10	10	6	..	..	..	..	..	..	10	10	6
1.....	13	5	3	36	20	28	10	7	6	68	39	37
2.....	16	9	9	10	6	6	11	3	3	37	18	18
3.....	..	..	..	7	2	2	10	4	1	17	6	3
4.....	27	9	1	20	5	1	26	6	1	73	20	3
5.....	24	1	1	36	1	1	27	2	2	87	4	4
6.....	17	2	0	8	1	0	17	0	0	42	3	0
7.....	7	2	0	17	0	0	8	0	0	32	2	0
8.....	10	0	0	6	0	0	10	0	0	26	0	0
12.....	5	0	0	2	0	0	1	0	0	8	0	0
	138	36	20	142	44	38	129	22	13	409	102	71

set in considerably earlier. After 24 hours' exposure, of the 39 presumptive colonies removed from the D.-C. plates, 37 proved to be genuine typhoid. This percentage was maintained in equal ratio on the second day, but after this date fell rapidly, so that from the third to the fifth day of exposure, there could be found on cultures from each sac only one or two colonies that proved to be *B. typhosus*. After this date, 108 more colonies were taken off, but nearly all were eliminated by the litmus glucose test, and all proved negative typhoid on the application of the agglutination test.

TABLE 13.  
LONGEVITY OF *B. TYPHOSUS* EXPOSED TO THE DIRECT ACTION OF SEWAGE BACTERIA.

DAYS	SAC 15 (PARCHMENT)			SAC 16 (CELLOIDIN)			TOTALS		
	No. Col. Fished	No. Pres. Typh.	No. Proven Typh.	No. Col. Fished	No. Pres. Typh.	No. Proven Typh.	No. Col. Fished	No. Pres. Typh.	No. Proven Typh.
0.....	16	16	16	..	..	..	16	16	16
1.....	3	3	3	50	7	7	53	10	10
2.....	38	15	15	26	3	3	64	18	18
3.....	22	10	10	20	2	2	42	12	12
4.....	15	0	0	8	0	0	23	0	0
5.....	7	0	0	13	0	3	20	0	3
6.....	7	0	0	6	0	0	13	0	0
7.....	3	0	0	2	0	0	5	0	0
8.....	3	0	0	5	0	0	8	0	0
10.....	3	0	0	4	0	0	7	0	0
14.....	3	0	0	7	0	0	8	0	0
16.....	1	0	0	..	..	..	..	..	..
	118	44	44	141	12	15	250	56	59

*Series VI. Parchment and celloidin sacs filled with sewage and immersed in sewage.*—The same method of arrangement, as detailed in preceding series, was followed in this series, which was begun on August 15, 1905, and continued until

August 29. A parchment and a celloidin sac, heavily seeded with typhoid organisms (4,350,000 and 5,800,000 bacteria, respectively), were filled with fresh sewage and immersed as before in flowing sewage. Bacteriological examinations made with D.-C. media on the sewage showed usually a germ content ranging from 1,250,000-6,600,000. The chlorine content varied from 150 to 200 parts per million. The range in temperature was from 22° to 25° C. The results obtained are shown in Table 13.

In this series the disappearance of the typhoid organism was even more rapid than in the foregoing test. Two hundred and fifty-nine cultures were removed, and of these 59 proved to be typhoid. In the parchment sac none were found after the third day, while in the celloidin sac the presumptive typhoids were sparse after two or three days, and had entirely disappeared after five days.

These results are in harmony with those obtained the year before on the Chicago Drainage Canal, in that in both sets of experiments, the longevity of the typhoid organism was much shorter when exposed in sewage than in lake water. In the earlier series of studies, the introduced organism was not found after the third day, while practically the same result was obtained in both of the series here recorded, although scattering colonies were found on the culture plates as late as the fifth day.

Taking into consideration, the results obtained in both the Chicago series and those here described it would seem that the data obtained warrant the conviction that the typhoid organism is unable to retain its vitality as long when immersed in sewage as it does when in contact with lake water. This fact being determined, the next question of interest is to find the cause of this phenomenon. Is this diminished longevity due (1) to the direct action of the sewage bacteria themselves, or (2) to the by-products which this type of life produces? By means of the technical methods here used, it was thought that this problem might be solved by exposing the typhoid organisms in sacs filled with lake water to the influence of flowing sewage. If the period of longevity of the inoculated organism was fully as great when the lake water sacs were immersed in sewage as when subjected to the current of flowing lake water, then it would seem that the influence of the soluble growth products of sewage bacteria would be of no effect. On the other hand, if they died as quickly as they did in sewage, or nearly as soon, then it would appear that the soluble substances pass-

ing from the sewage outside through the permeable membranes exerted a harmful action.

To test this hypothesis, several series were instituted in which the inoculated organism was exposed in the way just mentioned.



## PART III.

## BACILLUS TYPHOSUS EXPOSED TO THE INFLUENCE OF DIFFUSIBLE SEWAGE BY-PRODUCTS AND TO WATER BACTERIA.

Three different sets of experiments were made on this point, in which sacs were filled with raw lake water, then inoculated with *B. typhosus* and the whole immersed in flowing sewage. Series VII, consisting of two sacs each of celloidin, parchment, and agar, was inoculated with 190,000–300,000 organisms per c.c. and run from May 2 to May 19 of this year.

Two other series (VIII and IX), each consisting of a celloidin and a parchment sac, were inoculated, one with a heavy seeding, the other with a light seeding, of *B. typhosus*, and immersed in flowing sewage.

*Series VII. Celloidin, parchment, and agar sacs, filled with lake water and immersed in flowing, fresh sewage.*—Six sacs were employed in this series, two each of the three types. The sewage on the outside of the sacs was fairly strong, as judged by sight and smell. It was quite turbid, and of a dark brown color, due to accumulation of organic matter, which collected on the walls of the containing reservoir, especially at and near the surface. A cover-glass preparation showed a matrix of colorless filaments, brownish cells, and amorphous matter. From time to time the germ content and the chlorine content of this flowing liquid was ascertained to gauge its relative condition. The bacterial counts were made after 24 hours' incubation. A longer period of incubation would doubtless have increased materially the figures given.

TABLE 14.  
CHARACTER OF SEWAGE AS TO CHLORINE AND BACTERIAL CONTENT.

	Chlorine Pts. per 1,000,000	Bacteria per c.c. Lactose Agar	Litmus Lactose Agar
May 2.....	...	128,250	Numerous acid and gas colonies
May 4.....	175	80,000	Gas formers and acid colonies abundant
May 5.....	...	...	...
May 6.....	125	61,500	Sewage forms abundant
May 8.....	22	160,000	Acid and gas colonies numerous
May 10.....	...	...	...
May 11.....	15	.....	.....
May 12.....	24	.....	.....
May 13.....	27	.....	.....
May 15.....	24	.....	.....
May 17.....	20	.....	.....
May 18.....	70	.....	.....

The variation in chlorine content was doubtless occasioned by the somewhat irregular addition of the urine to the sewage. The temperatures ranged in this series from 15° to 19° C., with an average temperature of 16.7° C. All three kinds of sacs were used in this series,

two sacs of each being employed. At the outset an attempt was made to determine the quantitative condition of the respective sacs in a manner similar to that indicated in Series I, but this was discontinued during the progress of the experiment when it became evident that a large proportion of the organisms found on the culture plates were not typhoid, but were water saprophytes. The germ content of the different sacs at the beginning ranged from 190,000 to 330,000 per c.c., which was, of course, mainly *B. typhosus*, as the water used in the sacs was lake water, containing not more than 150-300 bacteria per c.c. On the second and third days of exposure the total germ content in the different sacs had fallen 50-90 per cent, but after this date a marked increase occurred, reaching in the course of a few days several hundred thousand bacteria per c.c. It would appear from these data that the water bacteria originally introduced, with the lake water, into the sac, had multiplied extensively. This is a matter of import, as this multiplication might exert some effect upon the longevity of the pathogenic organism with which they were exposed.

A more important fact, however, is that this demonstrates the permeability of the sacs to substances from without, for this enormous development far transcends any growth that would have occurred without influx of organic matter from the outside. That none of this growth was due to the admission of the sewagic forms from the outside is conclusively shown by the entire absence of any gas forming acid colonies on all the plates for about two weeks. The colonies growing on the Drigalski-Conradi medium were uniformly of some shade of blue or grayish color. Not until the 13th day did any acid colony appear. On this day a single acid colony developed in cultures made from one of the parchment, and also from one of the agar, sacs. This condition was also observed the next day in the same parchment sac, and in the other agar sac. These results, therefore, speak strongly for the integrity of the sacs for a sufficiently long period, as they were surrounded continually with water containing hundreds, and often thousands, of acid and gas generating forms per c.c.

The records of the plates as to their colony appearance is herewith presented:

Date	Days of Exposure	Character of Culture Plates
May 2.....	0	All sacs cultured immediately after installation and found to contain very nearly a pure typhoid culture
May 3.....	1	Major portion of colonies typhoid on parchment (Sac 18); agar sac (Sac 22) very nearly a pure culture of typhoid
May 4.....	2	Presumptive typhoids apparently few on agar (Sac 21). Suspected colonies picked failed to grow
May 5.....	3	About one-half on celloidin (Sac 19) appear to be typhoid. Typhoid abundant on agar (Sacs 21 and 22)
May 7.....	5	Typhoid abundant on celloidin (Sac 19)
May 8.....	6	Typhoid quite numerous on celloidin (Sac 20)
May 9.....	7	Culture badly mixed. Suspected typhoids very sparingly present
May 10.....	8	Colonies on plates from agar sac appear negative typhoid
May 11.....	9	Several colonies on celloidin (Sac 19) resemble typhoid, but only one proves positive
May 13.....	11	All colonies evidently negative typhoid. Total colony count falling rapidly

The results obtained from the testing of the cultures fished from the culture plates are expressed in Table 15.

TABLE 15.

LONGEVITY OF B. TYPHOSUS IN PERMEABLE SACS FILLED WITH LAKE WATER AND EXPOSED TO INFLUENCE OF FRESH SEWAGE.

	SAC 17 PARCH.		SAC 18 PARCH.		SAC 19 CELL.		SAC 20 CELL.		SAC 21 AGAR		SAC 22 AGAR		TOTALS	
	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Typh. Col. Found	No. Col. Fished	No. Proven Typh.
1 hour.....	6	6	4	3	3	2	3	3	5	5	4	4	25	23
1 day.....	3	1	7	7	3	3	3	3	5	3	4	4	25	21
2 days.....	4	1	..	..	5	2	7	2	4	0	5	0	25	5
3 ".....	4	0	8	4	5	4	2	2	4	1	3	2	26	13
4 ".....	6	2	..	..	4	2	7	4	1	1	6	1	24	10
5 ".....	6	1	7	0	10	7	..	..	15	2	..	..	38	10
6 ".....	..	..	..	..	..	..	7	5	..	..	6	2	13	7
7 ".....	10	2	3	0	3	0	..	..	5	0	..	..	21	2
8 ".....	..	..	6	0	..	..	7	2	..	..	5	1	18	3
9 ".....	7	0	5	0	0	1	..	..	4	0	6	..	25	1
10 ".....	..	..	1	0	..	..	4	0	..	0	5	0	10	0
11 ".....	5	0	..	..	5	0	4	0	4	0	6	1	24	1
13 ".....	4	0	..	..	5	0	5	0	3	..	3	0	20	0
15 ".....	2	0	1	0	1	0	1	0	1	..	1	0	7	0
17 ".....	..	..	..	..	4	0	5	0	..	..	..	..	9	0
	57	13	42	14	57	21	55	21	51	12	48	15	310	96

In the parchment series, the character of the colonies in the culture plates had changed by the seventh day so that typhoid-like organisms were very sparingly present. The results in all of the sacs, as a whole were, however, more divergent than in any of the preceding series. An average of the whole six sacs used was something over seven days, not greatly different from those made in flow-

ing lake water. Even though the divergence in results was considerable, the sacs in this series showed a greater degree of longevity than in those in which the typhoid organisms were immersed in sewage. This would seem to indicate that the sewage in the outer reservoir exerted little or no effect on the vitality of the typhoid within. With such divergent results it is, however, impossible to draw any conclusion, and the further data on this point, presented in Series VIII and IX, are necessary as a basis for deductions.

*Series VIII. Celloidin and parchment sacs filled with lake water and immersed in flowing sewage.*—This series consisted of three sacs: one celloidin and one parchment, filled with raw lake water and inoculated with the usual typhoid strain; also, a third celloidin sac filled with lake water, but uninfected with the pathogenic organism. These three sacs were immersed in a bath of flowing sewage. The exposure was continued for a period of 14 days. The temperature ranged from 21° to 29° C., with an average of about 25° C. The sewage in the outside receptacle was fresh and quite strong. Chlorine determinations showed a range from 241 to 340 parts per million. The bacterial content of this outside sewage was usually from 3,500,000 to 7,000,000 organisms per c.c.

A bacterial determination on Drigalski-Conradi medium was made of the uninfected celloidin sac, and it is interesting to note the enormous development that occurred in the lake water submerged in the sewage, as shown in the following data.

TABLE 16.  
BACTERIAL CONTENT PER C.C. OF LAKE WATER IN CELLOIDIN SAC IMMERSSED IN FLOWING SEWAGE.

	Period of Im- mersion (Days)	Bacteria per c.c.	Acid Colonies
June 13.....	0	30	0
June 15.....	2	10,500	0
June 18.....	5	10,950,000	0
June 25.....	12	26,600,000	0

The above data are of importance, as showing the course of the changes that occur in the permeable sacs when immersed in a medium containing a large amount of organic matter. This sac filled with water showed a degree of growth that is almost unparalleled, multiplying in the course of 12 days nearly a million-fold. The fact that no acid colonies developed on these plates made from the water is proof of the integrity of the sacs. This bacterial growth is far in excess of that which occurs in the permeable sacs when immersed in flowing lake water, and would seem to be explained on the assumption that nutrient substances of a diffusible character are capable of passing through the membrane, from the sewage outside.



The record of the longevity of the typhoid type is shown below.

TABLE 17.  
LONGEVITY OF B. TYPHOSUS IN CELLOIDIN AND PARCHMENT SACS FILLED WITH LAKE WATER AND  
IMMERSED IN FLOWING SEWAGE.

	SAC 23 (PARCHMENT)		SAC 24 (CELLOIDIN)		
	No. Colonies Fished	No. Proven Typhoid	No. Colonies Fished	No. Presumptive Typhoid	No. Proven Typhoid
0 days.....	..	..	13	12	10
1 day.....	9	0			
2 days.....	6	0	9	0	0
4 ".....	13	5	10	8	8
6 ".....	18	9	Leak discovered, further sampling discontinued		
10 ".....	21	4			
11 ".....	22	0			
12 ".....	17	0			
13 ".....	15	0			
14 ".....	16	0			

In this series an accident happened to the celloidin sac. In some way a leak developed, which fact could of course be quickly detected by the appearance of red colonies on the D.-C. plates, whereas the lake water, to begin with, was free from all acid-producing forms. From Sac 23 it happened that no cultures were made between the 6th and the 10th days, so that the history at this point is not as complete as it should have been, but it is significant that the typhoid organisms were readily recovered on the 10th day. A large number of cultures were taken after this date (70 on four successive days), but in no case was any organism found that even simulated the typhoid type of colony or proved positive upon the application of the differential tests.

When these results are compared with those contained in Series VII, it appears in both series that the inoculated typhoid persisted for a considerably longer period than in case of direct contact with sewage itself.

A rapid change, as usual, took place in the general character of the culture plates. On July 14, the second day after the lake water was infected, the major portion of the plate cultures showed typical typhoid colonies. In the course of a few days the total germ content per c.c. of this sac increased rapidly, due to the growth of the water organisms. On the 17th, five days after starting the experiment, about one-third (16,000 organisms) of the total colony count was still *B. typhosus*. By the 19th this number had fallen to about 8,000 per c.c. On the 23d both D.-C. plates contained a few presumptive typhoids, but the later plates showed totally aberrant forms.

*Series IX. Celloidin and parchment sacs filled with lake water and immersed in flowing sewage.*—This series was started on August 15 and continued till the 29th. Two sacs, one of celloidin and the other of parchment, were inoculated lightly with

100,000 and 200,000 typhoid bacilli, respectively. The findings in this test are expressed in Table 18.

TABLE 18.  
LONGEVITY OF *B. TYPHOSUS* IN SACS FILLED WITH LAKE WATER AND EXPOSED TO THE ACTION OF  
FLOWING SEWAGE.

DAYS	SAC 25 (CELLOIDIN)			SAC 26 (PARCHMENT)			TOTALS		
	No. Col. Fished	No. Pres. Typh.	No. Proven Typh.	No. Col. Fished	No. Pres. Typh.	No. Proven Typh.	No. Col. Fished	No. Pres. Typh.	No. Proven Typh.
0.....	12	12	12	14	14	13	26	26	25
2.....	12	2	2	12	1	1	24	3	3
4.....	17	0	0	21	1	1	38	1	1
6.....	26	11	11	30	11	11	56	22	22
7.....	10	6	6	59	14	14	60	20	20
8.....	21	0	0	31	1	1	52	1	1
10.....	3	0	0	5	0	0	8	0	0
13.....	7	0	0	9	0	0	16	0	0
14.....	7	0	0	12	0	0	19	0	0
	115	31	31	193	32	41	308	53	72

The most evident change in this series came suddenly on the eighth day. In the celloidin sac no typhoids were found on this date, or at any subsequent time, and in the parchment sac only one typhoid colony appeared on the eighth day, and none thereafter.

The results of these three foregoing series (see Summary, Table 19) show some variation in the longevity of the typhoid organism, but not more than was observed in the earlier series, where the exposure was in lake water alone. In general the typhoid bacillus persisted in these cases for about seven or eight days, with an occasional instance where vitality was prolonged for 10 or 11 days. These results stand in striking contrast to those obtained in the sewage series. This variation in longevity is brought out under optimum conditions in the case of two of the series that were run under conditions identical except as to the nature of the liquid in the sac.

Series VI and IX were immersed in the same sewage and run at the same time. Series VI contained sacs filled with lake water, while in Series IX the sacs were filled with sewage. The sewage series also received much the heavier seeding. As to results, the typhoid organism died in the sewage sacs in from three to five days, while in the sac filled with lake water, but immersed in same stream of sewage, it lived seven to eight days.

To permit of more ready comparison of results, reference can be made to Table 19, in which is summarized the results of all of the

experiments made on the sewage series and those in which sacs were filled with lake water and immersed in sewage.

TABLE 10.  
SUMMARY OF EXPERIMENTS WHERE EXPOSURE WAS MADE IN SEWAGE SACS, ALSO IN LAKE WATER  
SACS IMMERSSED IN FLOWING SEWAGE.

		SERIES	SAC No.	KIND OF SAC	DAYS														TOTAL No. COL. FISHED	No. PROV'N TYPHOID COL.
					0	1	2	3	4	5	6	7	8	9	10	11	12	13		
Sewage sacs	V	12	Agar.....	+	+	+		+	+	0	0	0			0					
		13	Parchment.	+	+	+		+	+	0	0	0			0					
		14	Celloidin ..	+	+	+	+	+	+	0	0	0			0			400	71	
VI	15	Parchment.	+	+	+	+	0	0	0		0		0		0	0		250	50	
	16	Celloidin ..	+	+	+	+	0	0	0		0		0		0	0				
VII	17	Parchment.	+	+	+	0	+	+		+		0	0	0	0	0				
	18	Parchment.	+	+	+	+	+	+		+	0	0		0	0	0				
	19	Celloidin ..	+	+	+	+	+	+			0	+	+	0	0	0				
	20	Celloidin ..	+	+	+	+	+	+	+		+		0	0	0	0				
	21	Agar .....	+	+	+	+	+	+		0		0		0	0	0				
	22	Agar .....	+	+	0	+	+	+	+		+		0		+	0	0	310	96	
VIII	23	Parchment.		0	0				+	lea	k'd	disc	ove	+	0	0	0	160	36	
	24	Celloidin ..	+	+	+		+		+					0	0	0				
IX	25	Celloidin ..	+		+		0		+	+	+	0				0	0	308	72	
	26	Parchment.	+	+	+		+		+	+	+				0	0				
																		1,455	334	

The evident conclusion which these data support is that the results obtained in the three series (VII-IX) in which the sacs were filled with typhoid-infected lake water and immersed in sewage are much more nearly in accord with the series exposed to running water (I-IV) than to those subjected to the direct action of sewage (V-VI). This would seem to indicate that the more destructive influence of sewage was not exerted unless the typhoid organism was in intimate contact with the sewage bacteria themselves.

*Series X.* One more possible combination existed, which was tried in order to test all aspects of the question at issue. Sacs filled with water had been immersed in flowing water; those filled with sewage had been placed in a sewage bath; and others filled with water had also been subjected to the influence of flowing sewage. The remaining combination of sewage-filled sac immersed in flowing water was therefore tried. In this case only a single sac was used (parchment). It was heavily inoculated with typhoid, 4,350,000 organisms per c.c. of a 24 hour culture.

A rapid death of the introduced germ occurred in this sac in the course of a few days. The latest recovery was on the fifth day, although even prior to this it had been greatly reduced in numbers. This result is practically the same as the sewage sacs immersed in

TABLE 20.  
LONGEVITY OF *B. TYPHOSUS* IN PARCHMENT SAC FILLED WITH SEWAGE AND IMMERSSED IN WATER.

DAYS	SAC 27 (PARCHMENT)		
	No. Colonies Fished	No. Pres. Typhoid	No. Proven Typhoid
0.....	20	10	10
1.....	2	2	2
2.....	30	6	6
3.....	18	2	2
4.....	12	0	0
5.....	12	1	1
6.....	4	0	0
8.....	0	0	0
10.....	5	0	0
13.....	17	0	0
14.....	9	0	0
	129	30	30

sewage. Although undoubtedly considerable diffusion of soluble substances would occur in this case, no material variation in the longevity of the pathogenic organism was observed. This is in accord with all of the previous results, and indicates that the longevity of *B. typhosus* is diminished when the organism is in direct contact with sewage bacteria.

In Table 21 are compiled the results of all experiments, expressed in days. This signifies the maximum period of time in each experiment during which the typhoid bacillus could be recovered. For findings other than the end results, comparative summaries may be found in Tables 10 and 19.

TABLE 21.  
SUMMARY OF RESULTS.

B. TYPHOSUS EXPOSED TO	SACS IMMERSSED IN	SERIES No.	LONGEVITY OF TYPHOID (IN DAYS)			
			Agar	Celloidin	Parchm't	Glass
Lake water.....	Lake Water....	1	8, 8	8, 10		13
		2	8	8		
		3		6	6, 8	
		4		10		
Sewage.....	Sewage.....	5	5	5	5	
		6		5	3	
Lake water.....	Sewage.....	7	5	8, 9	3, 7	
		8		?	10	
		9		7	8	
Sewage.....	Lake water.....	10			5	

### CONCLUSIONS.

1. Three types of permeable sacs (celloidin, parchment, and agar films) were employed to hold the typhoid organism imprisoned while it was exposed to the influence of water and sewage bacteria.



2. Tests made with chlorides, sugar, and peptone indicate that these sacs were readily permeable, while other tests demonstrated that they were wholly germ-tight.

3. In four series of examinations where *B. typhosus* was exposed to the action of flowing lake water (Mendota), the longevity of the organism ranged from 8 to 10 days, agreeing quite closely with the experiments previously reported on Lake Michigan water under similar experimental conditions.

4. Where *B. typhosus* was exposed directly to the action of sewage bacteria, its longevity was greatly diminished, three to five days being the longest time for which the organism could be recovered.

5. When the typhoid organism was exposed to the diffusible products of sewage bacteria, and not to the direct action of the organisms themselves, as was the case when the typhoid infected lake water-filled-sacs were exposed to flowing sewage, the longevity of the inoculated pathogenic form was increased. The results, although more variable than in either of the preceding cases, agreed more closely with those obtained in the lake-water series than with those exposed to the action of sewage itself. This would seem to indicate that the direct contact with sewage organisms was more detrimental to the vitality of the typhoid organism than the diffusible by-products of sewage forms.

6. Where typhoid-infected sewage-filled sacs were exposed to water, the longevity of *B. typhosus* was the same (five days) as where the bathing liquid was sewage. This again seems to indicate that the question of longevity is more dependent upon the actual contact with sewage types than it is upon contact with by-products capable of diffusion through these permeable membranes.

7. The uniformity noted in the results obtained in this investigation, and their confirmation of the work of the preceding year on the waters of Lake Michigan and the Chicago Drainage Canal would now seem to warrant the definite conclusion that the longevity of the typhoid bacillus in waters is materially affected by the germ content of its surroundings. In waters highly polluted with saprophytic bacteria, such as is the case in sewage, this disease organism is unable to survive for more than a few days (three to five in the experiments here described), a period of time materially shorter than that which is noted in normally unpolluted waters.

## ON THE RELATION BETWEEN OXYGEN IN WATER AND THE LONGEVITY OF THE TYPHOID BACILLUS.

GEORGE C. WHIPPLE AND ANDREW MAYER, JR.

THERE are many factors which affect the longevity of the typhoid bacillus in water. Experiments which we have made at various times during the last two years indicate that the presence of dissolved oxygen is one of the most important. Comparative experiments made by putting typhoid bacilli into two portions of water and maintaining one under anaerobic conditions, while the other remained oxygenated, indicated that the bacilli preserved their vitality for a much longer period when oxygen was present. This was found to be true also of the colon bacillus.

The experiments were varied in a number of ways, as shown by the following tables. The culture of typhoid bacillus, used in all the experiments, was obtained from Dr. Ezra Wilson of the Hoagland Laboratory. Before using it, it was submitted to preliminary cultivation in broth for 24 hours at 37° C. From the third generation in broth an agar streak culture was made, which was used in subsequent inoculations.

### EXPERIMENT I.\*

Brooklyn tap water was filtered through a Berkefeld filter, and 19 c.c. were put into test tubes and sterilized. To each of these tubes was then added 1 c.c. of a suspension of typhoid bacilli in water. Another series of tubes was prepared in a similar way, but the water was boiled to expel the dissolved oxygen. The tubes of this series were inoculated with typhoid bacilli as in the previous case.

The first set of tubes was placed in the 20° incubator; the second was also placed in the incubator, but was kept in a Novy jar in an atmosphere of hydrogen. After various periods of incubation, one tube of each set was withdrawn and samples were plated. The following table represents the results obtained:

\* Made with the assistance of Mr. Luther R. Sawin, Bacteriologist, Dept. of Water Supply, Gas, and Electricity.

DATE	PERIOD IN DAYS	TUBES KEPT IN AIR		TUBES KEPT IN HYDROGEN	
		Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
1903					
April 13.....	0	600,000	100.0	600,000	100.0
April 15.....	2	455,000	76.0	2,400	0.4
April 17.....	4	100,000	32.0	25	0.004
April 21.....	8	120,000	20.0	0	0.0
April 25.....	12	67,000	11.0	0	0.0
May 1.....	18	25,000	4.2	0	0.0
May 9.....	26	9,250	1.5	0	0.0
May 16.....	33	2,150	0.6	0	0.0
May 23.....	40	132	0.02	0	0.0
May 30.....	47	6	0.001	0	0.0
June 6.....	54	0	0.000	0	0.0

## EXPERIMENT 2.

The second experiment differed slightly from the first one. Sterilized distilled water was used instead of tap water, 150 c.c. was the quantity taken, and flasks were employed instead of test tubes. The water was not boiled. Carbonic acid was used in the Novy jar instead of hydrogen. The results obtained are given in the following table:

DATE	PERIOD IN DAYS	TUBES KEPT IN AIR		TUBES KEPT IN CARBONIC ACID	
		Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
1903					
July 18.....	0	400,000	100.0	400,000	100.0
July 20.....	2	265,000	66.0	110,000	27.5
July 22.....	4	.....	.....	1,500	0.38
July 26.....	8	50,000	12.5	0	0.0

## EXPERIMENT 3.

The experiment was made as above described except that an atmosphere of nitrogen, obtained by using pyrogallic acid and caustic potash, was used for the anaerobic flasks. The results are shown in the table following:

Period of Time	Kept in Air Number per c.c.	Kept in Nitrogen Number per c.c.
0 hours.....	3,120,000	3,120,000
24 ".....	No	0
48 ".....	record	0
72 ".....	kept	0

## EXPERIMENT 4.

This experiment was precisely like No. 3. The results obtained were as follows:

PERIOD OF TIME	TUBES KEPT IN AIR		TUBES KEPT IN NITROGEN	
	Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
0 hours.....	880,000	100	1,390,000	100
24 ".....	710,000	81	0	0

## EXPERIMENT 5.

This experiment was like No. 3 except that no control culture was made in air. The results were as follows:

PERIOD OF TIME	TUBES KEPT IN NITROGEN	
	Number per c.c.	Per Cent of Initial Number
0 hours.....	164,000	100.0
2 " .....	36,000	23.0
4 " .....	12,240	7.5
24 " .....	0	0.0

## EXPERIMENT 6.

This experiment differed from the preceding in that 1 c.c. of the broth culture was used to inoculate the water. This apparently produced a culture medium in which the bacilli could multiply. It was observed, however, that after multiplication had taken place the numbers fell off more rapidly in the anaerobic jar. The results were as follows:

Period of Time	Tubes Kept in Air Number per c.c.	Tubes Kept in Nitrogen Number per c.c.
0 hours.....	1,500,000	1,500,000
24 " .....	.....	3,000,000
7 days.....	7,200,000	4,000,000
21 " .....	5,000,000	550,000

## EXPERIMENT 7.

This experiment was conducted like the others, but *B. coli* was used instead of *B. typhosus*. The results obtained were as follows:

PERIOD OF TIME	TUBES KEPT IN AIR		TUBES KEPT IN NITROGEN	
	Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
Jan. 1-28, 1905				
0 hours.....	3,400,000	100	3,400,000	100.0
12 " .....	.....	.....	648,000	19.0
24 " .....	620,000	18	288,000	8.5
72 " .....	.....	..	10,500	0.3

If it be true, as indicated by these experiments, that the presence of dissolved oxygen in water causes typhoid fever bacilli to maintain their vitality for longer periods of time than when no oxygen is present, this throws some light on the results of various observations which have been made by other investigators. For instance, Jordan, Russell, and Zeit<sup>1</sup> found by their experiments on the waters of the Chicago Drainage Canal and the Illinois River that typhoid bacilli disappeared more quickly in polluted water than in relatively pure

<sup>1</sup> *Jour. Infect. Dis.*, 1904, 1, p. 641.



water. Among other reasons for explaining this fact, it is quite possible that the absence of oxygen in the polluted waters may have been an important influence. There seems to be some evidence that typhoid fever germs maintain their vitality more readily in the winter season than during the summer. It is worth noting in this connection that cold water contains much larger amounts of dissolved oxygen than does warm water. The effect of the septic tank on the longevity of pathogenic bacteria also involves the relation of oxygen

## THE RELATIVE APPLICABILITY OF CURRENT METHODS FOR THE DETERMINATION OF PUTRESCIBIL- ITY IN SEWAGE EFFLUENTS.

GEORGE A. JOHNSON, WILLIAM R. COPELAND, AND A. ELLIOTT  
KIMBERLY.

LESS attention was formerly paid to the question of the putrescibility of sewage effluents than is the case at the present time. For the most part the purification processes then embraced either broad irrigation fields and intermittent sand filtration, which normally yield effluents of a high degree of purity, or chemical precipitations which remove only about one-half of the total organic matter. To-day there are many sewage problems in which purification requires to be carried apparently only to the extent of obtaining a non-putrescible effluent. For this purpose coarse-grain filters have a wide field of usefulness, and they also are a factor to be considered as an intermediate treatment in those sections where porous, sandy soil is not available naturally, and where thorough purification is needed.

Contact filters, or sprinkling filters of broken stone, do not, of course, effect as high a degree of purification at their best as do filters of fine-grain material, and when unskillfully operated may yield effluents containing sufficient unstable organic matter to render them putrescible. To obtain the most satisfactory results from such processes, frequent data are required regarding the putrescibility of the effluent. The great need, therefore, as is universally recognized, is for a test, whereby a determination of the character of the effluent, so far as relates to its putrescibility, may be speedily made, and thus enable the results to serve as a direct guide in operating the plant.

The putrescibility tests now in general use, from a practical standpoint, possess a common weakness in that nearly all, if indeed not all, are based upon a method whereby the samples of the effluent require incubation for several days before the result can be definitely known. So far as they are of assistance to the operators of

sewage works, the results of such tests are of value only as matters of record, to serve as a general guide in future operating procedure.

Numerous attempts have been made to fix certain arbitrary standards of purity of sewage effluents by prescribing that an effluent shall not contain more than so much organic nitrogen, or oxygen absorbed, thus (the data expressed in parts per million)—the Rivers Pollution Commission require an effluent to be below organic carbon 20, organic nitrogen 3; Thames Conservancy, organic carbon 30, organic nitrogen 11; Derbyshire County Council, albuminoid ammonia 1, oxygen absorbed 10 (4 hours' test); Ribble Board, albuminoid ammonia 1, oxygen absorbed 20 (4 hours' test); Mersey and Irwell, albuminoid ammonia 1.4, oxygen consumed 14.<sup>3</sup> At this time, however, there is an almost unanimous feeling against even the limited applicability of such standards. Albuminoid ammonia, organic nitrogen, and oxygen consumed indicate, not the exact proportion of putrescible matter in a sample, but rather, generally speaking, only a component part of these substances. Nitrogen, as determined by these tests, may be derived both from stable and unstable matters; organic carbon, as indicated by the oxygen consumed tests, may be represented not only by putrescible carbonaceous organic matter, but also by certain non putrescible substances requiring oxygen for their oxidation.

The putrescibility of a sewage effluent, as is quite generally recognized at this time, is intimately related to its composition with respect to its relative content of stable and unstable organic matter, readily oxidizable mineral substances, and the relative abundance of available oxygen present. For this reason it is certain that the mere knowledge of the amounts of putrescible organic matter present in a sample would be insufficient evidence upon which to base an opinion of the putrescibility of the sample. A knowledge of the amount of available oxygen present to counteract the putrescible tendencies of the sample is required. With a fairly accurate knowledge of the oxygen consumed by, and the oxygen available in, a sample, it is probable that in some cases at least it may be possible to fix arbitrary standards of purity of sewage effluents, so far as their putrescibility is concerned.

## INCUBATOR TESTS FOR THE DETERMINATION OF PUTRESCIBILITY.

*Brief historical résumé.*—The first recorded test for the character of sewage polluted water, so far as we are aware, was proposed in 1870 by Heisch,<sup>1</sup> who noted the fouling and growths produced when the sample, mixed with cane sugar, was exposed to sunlight. In 1884, Dupré<sup>2</sup> stated that when sewage polluted water was kept for 10 days out of contact with the air, a more or less complete absorption of the dissolved oxygen would take place, and that by a determination of the dissolved oxygen before and after incubation, an idea might be obtained regarding the amount of organic matter present. This process, as pointed out by Rideal,<sup>3</sup> aimed at determining the number of organisms present in the sample, rather than the amount of putrescible compounds contained therein.

Scudder,<sup>4</sup> chemist to the Mersey and Irwell Joint Committee, introduced in 1895 the incubator test, which, subject to a number of modifications, is in more or less general use at the present time. At the inception of this test, the procedure simply consisted in completely filling a bottle with the sample, stoppering tightly, and incubating for a few days at summer temperature. Later<sup>5</sup> Scudder refined the method by including in the technique the determination of the oxygen absorbed in cold acid permanganate in three minutes, before and after an incubation period of from five to six days at 75° F. This method was also used by Clowes<sup>6</sup> in the London experiments, and by Fowler<sup>7</sup> at Manchester. In the early Manchester experiments<sup>8</sup> this test was modified by the use of a period of incubation of from six to seven days at 80° F. Scudder<sup>9</sup> later included the determination of dissolved oxygen and nitrates, before and after incubation, since he found that effluents which did not show an increase in the amount of oxygen absorbed from permanganate would also contain after incubation residual amounts of dissolved oxygen and nitrates.

Ross<sup>10</sup> incubates at 80° F. for seven days, and further estimates the degree of putrescibility of the samples by determining the oxygen absorbed from permanganate in 15 minutes and four hours, respectively, at a temperature of 80° F. This method is also used by Wilkinson,<sup>11</sup> at the Oldham Sewage Works. Stoddart's<sup>12</sup> modification of the incubator test aimed to place the determination of the odor of putrescible samples upon a more severe basis, by making a simple quantitative test for hydrogen sulphide. Clark<sup>13</sup> incubates the samples at 80° F. for two to five days, and determines the oxygen consumed from acid permanganate during a two-minute boiling period, before and after incubation.

Attempts have been made in a number of instances to place the incubator test on a more practical basis, by imitating in the laboratory conditions such as obtain when the effluent is discharged into the watercourse. This was effected by diluting the sample with varying volumes of river water, corresponding to the dilution obtainable in practice, following which the sample was incubated at summer temperature for a number of days. Thus Adency<sup>14</sup> suggests the dilution of the sample with river water before incubation. At Manchester,<sup>15</sup> it has been the practice for some time to incubate mixtures of the effluents and canal water. Clark<sup>16</sup> mixes equal volumes of effluent with tap water, and determines the amount of dissolved oxygen before and after incubation for five days at 80° F. In conjunction with this test he uses the two-minute oxygen consumed test already referred to, together with determinations of the amount of nitrites and nitrates present before and after incubation. Dunbar and Thumm<sup>17</sup> incubate the sample for one week in closed flasks at 68° F., note the formation of odors, and determine the presence of hydrogen sulphide with lead acetate paper.



The above is a necessarily somewhat incomplete digest of published literature bearing upon the incubator test for the determination of putrescibility. As is generally recognized, all of these tests, while yielding results of definite value, fall short of the most practical requirements, since the results obtained by these methods are not immediately available for the guidance of the sewage works operator.

*Results of the studies at Columbus on the incubator test.*— In a critical study of the incubator test and its various modifications at the Columbus Sewage Testing Station, we first turned our attention to a means of reducing the period of incubation. By adopting a temperature of 37° C., we were able in a majority of cases to obtain the same results as when, at lower temperatures, periods of incubation of several days were employed. Many determinations indicated that if odors did not develop after 24 hours at 37° C., they would also not be apparent after five days at 27° C. The relation between the period and the temperature of incubation in effecting putrefactive changes is clearly shown in the following table:

TABLE 1.  
EFFECT OF TEMPERATURE AND PERIOD OF INCUBATION ON THE RAPIDITY OF THE DEVELOPMENT OF PUTRESCENCE IN SEWAGE EFFLUENTS.

Character of Device	Coarse-Grain Filter		High Rate Sand Filter	
Temperature of incubation, deg. C. ....	27	37	27	37
Period of incubation, days .....	5	1	5	1
Number of samples which gave positive results .....	117	108*	21	18*
Per cent which positive results obtained at 37° C. were of those obtained at 27° C. ....	92		86	

\*The balance of the samples incubated at 37° C. gave positive results at the end of 48 hours.

Our studies of the incubator test at Columbus, as outlined above, have shown that the period of incubation may be advisedly reduced to 24 hours, provided a temperature of incubation of 37° C. (98.5° F.) be used, since about 90 per cent of the samples incubated at 48 hours at this temperature developed odors of putrefaction in 24 hours.

#### SPECIAL CHEMICAL TESTS FOR DETERMINING THE PRESENCE OF PUTRESCIBLE COMPOUNDS IN SEWAGE EFFLUENTS.

Attempts have been made to determine by direct chemical tests the putrescibility of sewage effluents. Spitta<sup>18</sup> suggests that by adding to the sample enough methylene blue to impart a faint color, putrescible samples will quickly and completely discharge the blue color when incubated for a few hours at a temperature of 72° to 79° F. Fowler<sup>19</sup> has suggested that this test has quantitative possibilities which have yet to be worked out.

In the Columbus studies, consideration was given to this test, as well as to others, whereby the presence of albuminous and proteid

matters might be directly determined. From a study of the methylene blue test, we have found that oftentimes there would be present substances other than putrescible organic matters, such as sulphide of iron and hydrogen sulphide, which would immediately discharge the blue color, before the putrescible matters were able to act.

The other tests studied in this connection included the biuret reaction<sup>20</sup> and the use of Millon's reagent,<sup>21</sup> whereby it was thought that the presence of undecomposed albuminous substances could be detected. After an extended trial it was found that these tests did not distinguish between putrescible and non-putrescible substances; chiefly, it is thought, because putrescible substances are not confined wholly to the albuminoid class.

In addition to these studies, attempts were made to determine the putrescibility of an effluent by measuring its propensity to absorb oxygen from chemical oxidizing agents. First among these, iodine and hydrogen peroxide were studied. While it is true that, in connection with the use of all chemical oxidizing agents, certain complications arise in the presence of inorganic reducing agents, iodine and hydrogen peroxide are reagents of such high sensibility, that there are many inherent practical difficulties encountered in their use, which bar them from serious consideration among tests for determining the propensity of the effluent to consume oxygen.

In the further work at Columbus, a method was sought for the determination of putrescibility, which would correlate the results of the regular chemical analysis and the putrescible properties of the effluent. The direction in which the solution of the question seemed to lie, as has been previously noted, was in the balance between the oxygen available to offset the putrefactive tendencies of the effluent, and the actual amount of oxygen consumed in effecting this result. It was considered that in a certain degree the amount of oxygen consumed could be referred to the regular "oxygen consumed" values from permanganate. Where the amount of available oxygen exceeded the amount of oxygen consumed, it appeared certain that such conditions favored the ultimate complete oxidation of the unstable matters without accompanying nuisances.

In order to obtain information on the relation between the results obtained by the various "oxygen consumed" tests, coincident

with the putrescibility of the sample as referred to the odor developed after incubation in a tightly stoppered bottle for 24 hours at 37° C., the several tests were applied to a large number of samples collected from various sources. The modifications in the oxygen consumed test which were used in this comparative study were as follows: (a) The instantaneous oxidation by permanganate in a cold acid solution;<sup>22</sup> (b) Oxidation by permanganate during a three-minute period of digestion in a cold acid solution;<sup>23</sup> (c) Ditto in a 15-minute period of digestion;<sup>24</sup> (d) Oxidation by permanganate during a period of digestion of 30 minutes in boiling water;<sup>25</sup> (e) Oxidation by permanganate at boiling temperature during a five-minute period.<sup>26</sup> Duplicate samples were also incubated, as above described, and their putrescibility determined by the odor test. The results, which are given in the following table, indicate that the relative amounts of "oxygen consumed," as shown by the modified methods according to which the more rapid determinations are made, are naturally greater in the putrescible than in the non-putrescible samples. The more prolonged and higher temperature tests simply serve to emphasize these differences.

TABLE 2.  
COMPARISON OF OXYGEN CONSUMED RESULTS OBTAINED BY DIFFERENT METHODS OF ANALYSIS IN  
PUTRESCIBLE AND NON-PUTRESCIBLE SAMPLES.

Method	Time of Contact (Minutes)	Tempera- ture, Deg. F.	Oxygen Consumed Parts per Million		Relation of Results by Other Methods to Those Obtained by the Boston Method	
			Putres- cible Samples <i>a</i>	Non- Putrescible Samples <i>b</i>		
					<i>a</i>	<i>b</i>
Immediate	1	80	3.5	1.5	0.17	0.21
English	3	80	4.2	1.4	0.20	0.20
English	15	80	9.7	2.5	0.46	0.35
Boston	5	Boiling	20.9	7.2	1.00	1.00
Palmer	30	"	44.7	12.0	2.13	1.67

#### RELATION OF THE COMPOSITION OF A SEWAGE EFFLUENT TO ITS STABILITY.

*Unstable organic and inorganic matter.* — Putrescibility may be due to the presence of complex bodies of either animal or vegetable origin, depending entirely upon the character of the raw sewage. The relative amounts of these two classes of organic matter are roughly indicated by the amounts of organic nitrogen, and "oxygen

consumed," respectively. Since the same amounts of nitrogen or oxygen consumed may be present in one case in a crude sewage, and in another in a stable effluent, it is clear, as pointed out by Dunbar and Thumm,<sup>27</sup> that it is the relative, and not the absolute, amount of organic matter in sewage before and after purification, that is to be taken as the criterion for the consideration of questions relating to putrescibility.

The breaking-down by hydrolysis of the complex, highly organized bodies containing sulphur gives rise to the formation of considerable amounts of sulphureted hydrogen. Sulphur in this form rarely occurs in sewage effluents, however, since the iron in the crude sewage, or in the filtering material, combines with the sulphureted hydrogen to a sulphide, which is ultimately oxidized to sulphate in effluents which are normally stable. In fact, the absence of sulphide of iron in the effluent<sup>28</sup> may be taken as an indication of the adequacy of the aeration facilities within the filter; and further, perhaps of more moment for the putrescibility question, sulphide of iron will not develop in an effluent when stored, provided the conversion to a stable form of the organic matter therein has been sufficiently completed in the filter. The putrefactive tendencies of a sewage effluent thus refer to unstable organic bodies which are generally found to be coincident with the presence, or the subsequent formation, of sulphide of iron.

#### THE AVAILABILITY OF OXYGEN OF DIFFERENT KINDS AS FOUND IN SEWAGE EFFLUENTS.

In the case of some of the rapid processes, a very considerable proportion of the purification effected by rapid filters of coarse material may be said to take place under anaerobic conditions, and through agencies in which oxygen is not directly concerned. But, as has already been mentioned, the great majority of causes underlying putrescible conditions in sewage effluents refer to a supply of oxygen within the filter insufficient to effect the changes which bring about stable conditions in the effluent.

*Availability of gaseous oxygen and oxygen combined with nitrogen.* — In a sewage effluent there are two sources of oxygen, which the studies of a number of workers besides ourselves have shown



to be available for the protection of the effluent against putrefaction. These are dissolved gaseous oxygen, and the oxygenated compounds of nitrogen, nitrogen pentoxide ( $N_2O_5$ ), and nitrogen trioxide ( $N_2O_3$ ), commonly spoken of as nitrate and nitrite oxygen, respectively. Rideal<sup>29</sup> says that the "available oxygen" is that present as nitrate and nitrite, and that the amount in a satisfactory effluent is quite sufficient to overcome putrefaction, without the aid of the dissolved oxygen in the stream into which the effluent is discharged. By adding certain amounts of sodium nitrate to clarified sewage, Adeney and Scott-Moncrief<sup>30</sup> take advantage of the availability of oxygen from that source. Sewage so treated, after retention in a tank for several hours, may be discharged therefrom in a stable condition. During the experiments at Manchester,<sup>31</sup> the effect of mixing a nitrated and a crude tank effluent was studied as a means of reducing the required acreage of the purification works. The result of these studies showed that, within certain limits, a considerable volume of crude tank effluent could be rendered stable in this manner. In experiments in which equal volumes of tap water and putrescible sewage effluent were kept at summer temperature for several days, Clark<sup>32</sup> showed a marked diminution in the initial amounts of dissolved oxygen and nitrate oxygen. In non-putrescible samples, treated similarly, no such pronounced reduction in the available oxygen took place, and an appreciable amount of residual oxygen was always noted. Fowler<sup>33</sup> also affirms that a well-nitrated effluent is protected against putrefactive tendencies. The well-known researches by Gayon and Dupetit<sup>34</sup> show that marked decomposition of unstable organic compounds is effected by the reduction of nitrates by bacteria. Similar observations have been made by numerous other observers.

*Availability of other sources of oxygen.* — There are a number of oxygenated compounds in sewage effluents, besides those already discussed, which require a passing comment regarding the availability of oxygen from such sources for overcoming putrescible conditions. Sulphates and oxide of iron are among those which particularly suggest themselves. The question arises, however, whether we are to regard oxygen, chemically combined in such stable atomic aggregations, as available under the reduction forces at work when

a but partially purified sewage undergoes putrefactive change. While the bacterial reduction of sulphate to sulphide, and of ferric oxide to its ferrous state, has been noted by other observers, the conditions were somewhat different from those encountered in studies relating to the putrescibility of sewage effluents. Clark<sup>41</sup> is of the opinion that sulphates in sewage will undergo a considerable decomposition under septic conditions, the extent of their reduction in a septic tank, in which a period of flow of about 24 hours was maintained, being shown as about 7 per cent. Fuller<sup>42</sup> has shown that in the sediment deposited from Ohio River water, prior to the process of the decomposition of the organic matter contained therein, it is probable that there took place a reduction of the oxygen present in the mineral compounds, such as sulphates and nitrates, and a reduction of ferric to ferrous iron.

During the studies on the putrescibility question made at the Columbus Sewage Testing Station, such question as these have been given a careful consideration. For the purpose of learning whether any considerable reduction of the sulphates, which the Columbus sewage normally contains, took place under the active reducing actions present in septic tanks, the influent and the effluent of two of the septic tanks in operation at the testing station were sampled at half-hourly and hourly intervals, respectively, for a period of one week during the month of May. At the end of that time the various portions were mixed and examined for sulphates. The results of this experiment are presented in Table 3 and show conclusively that sulphates ( $\text{SO}_4$ ), present in the applied sewage to the extent of 200 parts per million, did not suffer any appreciable reduction when exposed for 16 and 24 hours, respectively, to the reducing action incidental to the septic process under local conditions.

While, as Fuller<sup>42</sup> has pointed out, it is probable that oxide of iron will yield up its oxygen under the conditions stated, its reduction implies highly putrescent conditions such as would not obtain in an effluent of ultimate stability. It seems clear, therefore, that in overcoming putrescible tendencies in a sewage effluent, only gaseous oxygen and oxygen combined with nitrogen can be considered as available sources of oxygen in effecting bacterial oxidation to a condition of ultimate stability. Further, as already noted, fixed

TABLE 3.

TABLE SHOWING THE EFFECT OF SEPTIC ACTION UPON THE SULPHATES IN CRUDE SEWAGE.

DEVICE	PARTS PER MILLION $\text{SO}_4$		
	1	2	Average
Crude sewage.....	208	208	208
16 Hour septic tank .....	203	205	204
24 Hour septic tank .....	201	...	201

NOTE. — Method of determining sulphates: In boiling acidified solution precipitated sulphate with barium chloride. Filtered through tarred Gooch crucible, ignited, and weighed.

standards referring to certain maximum permissible amounts of organic nitrogen, albuminoid ammonia, or oxygen consumed, cannot be taken as indications of such conditions.

#### METHODS FOR THE DETERMINATION OF PUTRESCIBILITY BASED UPON CURRENT CHEMICAL DATA.

With a thorough knowledge of all the factors associated with the causation of putrescibility, it would seem that the results of chemical analysis which, up to the present time and now, represent the extent to which the differentiation of the several constituents in the effluent may be carried, should furnish also information as to the putrescible properties of a given sample. Considering introspectively the nature of the conditions under which putrescibility takes place, and studying carefully also the factors which are instrumental in preventing the putrescent reorganization of unstable organic matter, we are forced, like others, to the conclusion that the putrescibility factor narrows itself down to the amount of oxygen available for aerobic decomposition.

The oxygen consumed results, as obtained by the standard five-minute boiling method,<sup>26</sup> measure fairly accurately the amount of oxygen consumed in the oxidation of the unstable matter present in the sample. Since the reactions, as a result of which the ultimate reorganization of crude organic matter takes place under aerobic conditions, are subject to the uncertainties incidental to the action of bacteria, it would seem inadmissible to infer that the amount of oxygen necessary to complete the oxidation of the putrescible organic constituents of the sewage effluent, as indicated by chemical oxidizing agents, would be the same as that necessary for the accomplishment of the oxidation of such matter by bacteria. It seems certain,

therefore, that the most probable direction in which would lie the satisfactory interpretation of "oxygen consumed" results, with respect to the premises of putrescibility, refers to the establishment of a relation between the oxygen absorbed from permanganate and its bacterial equivalent.

The oxygen available in a sewage effluent to offset its putrescible tendencies, as has already been pointed out, is the amount of oxygen present in the sample in a dissolved gaseous state and that combined with nitrogen in the form of nitrate and nitrite. A measure of the putrescible tendencies of an effluent may be closely estimated from the amount of oxygen absorbed from permanganate during a three-minute digestion of the sample in a cold acid solution. This feature has been put forward many times before by other workers, and it is merely our desire at this time to record the fact that our work confirms the stability of their conclusions. We desire to emphasize the fact, however, that in some instances this may not be absolutely true, but for the majority of cases we believe that such is the case. Further, the results of our studies lead us to feel that under average conditions there is an intimate relationship existing between the three-minute cold test,<sup>23</sup> and the five-minute boil test.<sup>26</sup> In Table 2 it was clearly shown that the former value was one-fifth of the latter.

It is the available oxygen contained in the effluent in the form of dissolved oxygen and oxygen combined as nitrates and nitrites, which may be said to serve as a protecting agent in preventing the establishment of putrescent conditions. Our studies indicate that the existing relation between the oxygen absorbed from permanganate in cold acid solutions in a period of three minutes—or a similar "oxygen consumed" value obtained by dividing the result of the five-minute boil test by five—and the available oxygen which the effluent contains, may furnish a satisfactory means whereby the oxygen required and available, respectively, may be placed upon a comparable basis.

RELATIVE AVAILABILITY OF DISSOLVED OXYGEN AND OXYGEN  
COMBINED WITH NITROGEN IN THE NEUTRALIZATION OF  
PUTRESCIBLE TENDENCIES IN SEWAGE EFFLUENTS.

In our studies of the putrescible qualities of sewage effluents, and those factors which serve to prevent putrescence, certain facts



were brought out with considerable distinctness. We desired to learn if a fairly definite relationship existed between the consumed and the available oxygen, as indicated by the results obtained through the medium of certain "oxygen consumed" methods, and by the amounts of oxygen contained in the effluent which could be properly considered as available for the prevention of putrescent conditions. A large number of tests were made to determine the "oxygen consumed" by the three-minute cold and the five-minute boil methods, respectively, together with the amount of available oxygen contained in the effluents of the various devices under study at the Sewage Testing Station. The results of these studies brought out the fact that there does exist a more or less definite relation between the amount of oxygen consumed, as determined by chemical tests, the dissolved oxygen, and that available through bacterial action from the nitrates and nitrites which the effluent contains. In the course of these studies, carried on side by side with incubator tests, we were inclined to believe that in cases where the oxygen consumed, as shown by the chemical tests described above, was, before incubation, less than the amount of available oxygen computed from the amount of dissolved oxygen and from those amounts available from nitrites and nitrates, respectively, such an effluent would not putrefy on incubation; and such, in the majority of cases, has turned out to be the case.

To place such deductions as these on a stable basis, we realize that a definite statement is required as to the respective coefficient of availability of oxygen dissolved in the effluent in the free gaseous form, and that present in combination with nitrogen in the form of nitrate and nitrite. In this connection we have already noted that sprinkling filters yield at times a putrescible effluent which, while not well nitrified, may still contain amounts of dissolved oxygen sufficiently great to prevent the establishment of putrescent conditions, if the availability coefficient of dissolved oxygen were unity. That the reverse has been found to obtain in many instances is shown by the results presented in the next table. These data are representative of many similar cases, where, under the same conditions with respect to the content of the effluent in organic nitrogen and oxygen consumed, the effluent was clearly putrescent after in-

cubation. In these cases, while the nitrate and nitrite oxygen were present in low quantity, the amount of gaseous oxygen dissolved in the effluent was high. Dissolved oxygen plays such an important part in the disposal of sewage in flowing streams by preventing putrescent conditions therein that such results as these, in the absence of more extended evidence, are clearly to be regarded as abnormal. It is therefore the desire of the writers merely to suggest the possibility that such conditions may arise in the case of sprinkling filter effluents with the thought that perhaps further investigation under a different set of conditions may satisfactorily explain them.

TABLE 5.  
AVAILABILITY OF DISSOLVED OXYGEN.

PARTS PER MILLION					PUTRESCIBILITY TEST BY ODOR AFTER INCUBA- TION	OXYGEN CONSUMED (5 MIN. Boil+5) WAS TO OXYGEN AVAILABLE FROM:		
Oxygen Consumed			Available Oxygen			Dis- solved	Combined with Nitrogen	Total
5 Min. Boil+5	3 Min Cold	Dissolved	Combined with Nitrogen	Total				
7.6	6.5	7.1	4.1	11.2	+	1:0.93	1:0.54	1:1.47
6.6	6.3	3.5	5.2	8.7	+	0.53	0.79	1.32
6.0	6.0	5.2	2.4	7.6	+	0.87	0.40	1.27
5.6	5.8	5.7	1.6	7.3	+	1.02	0.29	1.31
6.0	5.5	7.3	1.5	8.8	+	1.22	0.25	1.47
5.2	5.7	6.6	3.1	9.7	+	1.27	0.60	1.87
5.8	5.5	8.3	2.0	10.3	+	1.43	0.35	1.78
5.4	6.0	8.4	2.5	10.9	+	1.56	0.46	2.02
5.0	5.5	8.3	2.7	11.0	?	1.66	0.54	2.20

In the following table the amount of oxygen consumed is represented both by the results of the five-minute boil method, and the three-minute cold method. In the computations of the amount of consumed oxygen the corrected results of the five-minute boil method are used, for the reason that it appears to us a somewhat more reliable method for indirectly measuring the amount of oxygen consumed by the easily oxidizable matter in a sewage effluent. The three-minute test, to our thought, possesses a considerable weakness in that it emphasizes disproportionately the value of inorganic compounds of ready oxidizability. Further, the technique entailed by this method is somewhat more delicate than that employed in the five-minute boil method, and subject to somewhat greater possibilities of inaccuracy.

TABLE 6.

SELECTED RESULTS OF PUTRESCIBILITY TESTS ILLUSTRATIVE OF THE VARIABLE VALUE OF THE SEVERAL AVAILABLE FORMS OF OXYGEN.

PARTS PER MILLION					Putresci- bility Test by Odor after In- cubation	OXYGEN CONSUMED (5 MIN. BOIL + 5) WAS TO OXYGEN AVAILABLE FROM:			Putrescent Condi- tions on Incubation Probably Due to the Inactivity of:
Oxygen Consumed		Available Oxygen				Dis- solved	Combined with Nitrogen	Total	
5 Min. Boil	3 Min. Cold	Dis- solved	Combined with Nitrogen	Total					
3.2	2.8	1.7	1.0	2.7	+	1:0.53	1:0.31	1:0.84	
4.2	3.9	7.4	7.0	14.4	?	1.70	1.67	3.43	
5.6	4.6	0.0	5.1	5.1	+	0.00	0.91	0.91	
7.2	9.2	6.2	0.9	7.1	+	0.86	0.12	0.98	
2.8	7.7	3.6	5.1	8.7	O	1.29	1.82	3.11	
3.2	4.5	0.1	2.5	2.6	+	0.03	0.78	0.81	
3.2	3.4	0.0	3.8	3.8	?	0.00	1.19	1.19	
5.0	3.8	7.7	2.7	10.4	?	1.54	0.54	2.08	
4.6	13.9	2.1	2.9	5.0	?	0.46	0.63	1.09	
4.2	6.8	1.6	4.9	6.5	?	0.38	1.17	1.55	
3.8	3.2	8.4	2.3	10.7	+	2.21	0.61	2.82	*
5.0	5.3	0.2	3.5	3.7	+	0.04	0.70	0.74	
0.8	3.7	6.4	8.0	14.4	+	0.65	0.82	1.47	*
4.0	3.4	7.9	6.8	14.7	?	1.97	1.70	3.67	
5.8	10.6	4.3	1.7	6.0	+	0.74	0.29	1.03	*
3.6	4.0	0.1	3.0	3.1	+	0.03	0.83	0.86	
7.2	8.8	5.3	5.5	10.8	+	0.74	0.76	1.50	*
4.4	4.2	6.7	4.1	10.8	?	1.52	0.93	2.45	
3.6	3.5	1.1	4.7	5.8	O	0.31	1.30	1.61	
5.2	5.0	3.6	1.4	5.0	?	0.60	0.27	0.96	
3.2	3.4	6.8	7.6	14.4	?	2.13	2.37	4.50	
3.2	3.6	0.0	5.8	5.8	O	0.00	1.81	1.81	
4.8	4.2	4.7	5.6	10.3	+	0.98	1.17	2.15	§
3.4	3.7	0.6	3.9	4.5	O	0.18	1.14	1.32	
2.2	2.8	0.1	2.1	2.2	?	0.05	0.95	1.00	
5.4	4.9	0.1	3.4	3.5	+	0.02	0.63	0.65	
5.4	6.3	6.3	2.5	8.8	O	1.17	0.46	1.63	
6.2	7.1	3.9	8.2	12.1	+	0.63	1.32	1.95	§
5.0	5.0	5.7	4.9	10.6	+	1.14	0.98	2.02	*
3.2	3.4	3.4	0.8	4.2	?	1.06	0.25	1.31	
4.2	3.6	2.5	3.2	5.7	O	0.60	0.76	1.35	
3.2	3.1	1.7	1.0	2.7	+	0.53	0.31	0.84	

NOTE. — + = Odor like  $H_2S$ , strong and lasting. ? = Ditto, but very faint and immediately disappearing. O = No offensive odor. \* = Dissolved oxygen. § = Oxygen combined with N.

#### THE RAPID ESTIMATION OF THE PUTRESCIBILITY OF SEWAGE EFFLUENTS.

From a practical standpoint no information regarding the efficiency of a sewage filter is so promptly required as is knowledge of the putrescibility of the effluent. The methods in use at this time for the determination of this feature generally include a period of incubation of from five to seven or more days. There is an urgent need, however, as is recognized by all, of a method which will permit of at least an approximation of the putrescible character of the effluent of a filter, and which will yield information sufficiently accurate to make possible opportune changes in operating proced-

ures whereby the character of the effluent may be improved at once, instead of several days after it has commenced to deteriorate. In the light of present knowledge on the subject, it would be unreasonable to expect a method to be found for this purpose which would, at all times and under all conditions, yield an absolutely correct result; but the results of our studies lead us to conclude that an idea of the putrescible character of a sewage effluent, sufficiently accurate for all ordinary purposes, may be secured in the space of an hour, or so, as follows:

*Determination of putrescibility by computation from the chemical data.* — The analytical data required include the "oxygen consumed" by the five-minute boil method, the nitrogen as nitrite and nitrate, respectively, and the dissolved oxygen. The oxygen consumed is estimated by dividing the "oxygen consumed" by five. This factor represents the coefficient whereby the "oxygen consumed" result may be converted to its bacterial equivalent, or the value indicating the amount of consumed oxygen. The amount of available oxygen is determined by converting the nitrogen, as nitrite and nitrate, respectively, to oxygen, by multiplying the nitrogen value of these two constituents by the factors 1.71 and 2.86, respectively, representing the relative amounts of oxygen combined with nitrogen in these two forms. The sum of the oxygen available from these nitrogen compounds, together with a somewhat uncertain proportion of the dissolved oxygen, represents the real active agent in the prevention of putrescent conditions in a sewage effluent.

With the data above outlined at hand, we feel that it is admissible to interpret the putrescibility of an effluent in the following manner:

First, when the consumed oxygen value is equal to or in excess of the amount of dissolved oxygen in the effluent, and no nitrates or nitrites are contained therein, the sample will putrefy.

Second, when the consumed oxygen value is equal to or slightly less than the amount of oxygen contained in the effluent in the form of nitrates, nitrites, and dissolved oxygen, the sample may or may not putrefy.

Third, when the consumed oxygen value is less than the oxygen contained in the effluent in the form of nitrates and nitrites, under ordinary circumstances the sample will not putrefy.



Such are the relations which our results indicate as existing between the constituents of chemical analysis and putrescibility. While we have found that such deductions, as outlined above, admitted of quite extended application to conditions obtaining at Columbus, we do not wish to be misunderstood as contending that these provisional criteria of putrescibility will prove to be of general applicability, under the great variety of conditions encountered in the practical control of sewage works. We feel, however, that the knowledge gained in these studies possesses considerable suggestive value, which may possibly serve as a basis for future studies along similar lines.

The chemical tests involved in the rapid estimation of putrescibility, as outlined above, are such that the services of a competent analyst will be required in connection with the sewage works where such tests may prove to be applicable. In small works, where the expense of such a man might be considered as prohibitive, the incubator tests may be employed to afford the desired information. In this connection we desire to call attention to the feasibility of employing a period of incubation of 24 to 48 hours at 37° C., instead of the longer periods at lower temperature in more or less general use at the present time.

In conclusion the writers desire to acknowledge the courtesy of Mr. Julian Griggs, chief engineer of the Board of Public Service, in permitting the use of data embodied in this paper. They desire further to make acknowledgment of the suggestions and criticisms offered by Mr. George W. Fuller, who, as one of the consulting engineers to the city of Columbus on sewage disposal matters, has closely followed the work herein described from its conception.

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# A COMPARATIVE REVIEW OF CURRENT METHODS FOR THE DETERMINATION OF ORGANIC MATTER IN SEWAGE.

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DURING the past five years, largely through the efforts of the Committee on Standard Methods of the American Public Health Association,<sup>1</sup> methods for the determination of organic matter in sewage have been placed upon a more definite and uniform basis than was formerly the case. The Committee, while specifying certain methods as standard methods, are yet cognizant of the considerable variability in the practice in different places in this country, and the difficulties attending radical changes in existing methods in the case of the older and long-established laboratories.

With the view of bringing these different methods together in concise form, it is the purpose of the writers to compare briefly the current practices in use in the different laboratories in this country, in England, and in Germany. The discussion will be confined to the Kjeldahl process, the determination of nitrogen as free ammonia, oxygen consumed, and loss on ignition.

## METHODS FOR THE DETERMINATION OF THE UNOXIDIZED NITROGEN.

*The Kjeldahl process.*—In the absence of a method whereby there may be obtained an accurate knowledge of the character and actual amount of the nitrogenous compounds which are normally present in sewage of domestic origin, the Kjeldahl process furnishes well-nigh indispensable information regarding the amount of nitrogen which occurs in sewage in complex atomic aggregations. This process, in use to some extent for a number of years, has finally become quite generally recognized as the most reliable means of determining the nitrogen of the organic matter, and as such has been recommended as a standard method for sewage work.

*Standard American method.*—The prescribed method for the determination of the total organic nitrogen in sewage embodies one or more of the salient features of the procedures used by Drown and Martin, Hazen,<sup>2</sup> and in England.<sup>3</sup> In the standard

method, the nitrogen as free ammonia is first removed by distillation with steam, following which the sample is digested with sulphuric acid until colorless; a small crystal of potassium permanganate is added, the digestate made alkaline with an excess of sodium carbonate, and finally diluted to a volume of 500 c.c. A suitable volume of this solution is then distilled with steam, and the ammonia evolved determined in the usual manner. Catalytic agents are not used in this method, and no steps are taken for the preliminary reduction of nitrified nitrogen.

*English methods.*—The methods of analysis described by Fowler,<sup>3</sup> by Rideal,<sup>4</sup> and employed by McGowan<sup>5</sup> may be taken as representative of English practices in sewage analysis.

McGowan and his associates have used the Kjeldahl process to a considerable extent to determine both the total unoxidized and organic nitrogen in sewage. Their methods include both distillation and a direct nesslerization of the neutralized digestate. Their practice in regard to the Kjeldahl process is essentially different from that in common use in this country, in that a reduction of nitrate nitrogen is considered necessary. This is effected by a very slow reduction with zinc and sulphuric acid, requiring five days for its completion. Their reports distinctly state that when the reduction is omitted, or when a shorter period than five days is employed, loss of nitrogen is inevitable.

A direct process described by them involves a neutralization of the digestate, and the addition of sodium oxalate for the removal of the calcium, following which a suitable portion of the settled solution is diluted and nesslerized. This method is not recommended by McGowan for general use, owing to the liability to turbid tubes, and to the necessity of preliminary reduction of nitrates before the actual Kjeldahl digestion is attempted. Rideal,<sup>4</sup> however, describes a direct method of which he thinks well, without commenting upon the necessity for preliminary reduction of nitrate nitrogen. The tediousness of McGowan's method obviously detracts from its value for practical routine work.

*German methods.*—From the works of Farensteiner, Buttenburg, and Korn,<sup>7</sup> and the reports of the Royal Bureau of Sewage Disposal at Berlin,<sup>8</sup> we learn that the Kjeldahl process is there regarded as of great value in sewage work. The official method includes the destruction of all oxidized nitrogen by treatment with sodium sulphite, ferric chloride, and dilute sulphuric acid, after which follows the usual digestion, neutralization, and distillation. Owing to the high concentration of German sewages the final step is a distillation into standard acid rather than nesslerization. In some cases catalytic agents are used, such as platinic chloride and copper oxide,<sup>9</sup> or copper sulphate or oxide.<sup>10</sup> The German method of preliminary reduction is much more satisfactory than the tedious English method. Neither of these, however, is considered essential for American conditions.

*Phelps' method.*—In the work at the Sewage Experiment Station of the Massachusetts Institute of Technology, Phelps<sup>6</sup> has used a Kjeldahl method wherein copper sulphate is employed as a catalyzing agent and permanganate is added at the completion of the digestion. Phelps shows very clearly the necessity for carrying the digestion to a point where a colorless solution will result, and brings forward data as to the feasibility of using permanganate; wherefrom he concludes that permanganate, although not essential in all cases, must still be considered a necessary adjunct to the reagents used in the Kjeldahl process as employed by Palmer.<sup>11</sup> He concludes his paper with suggestions in favor of the feasibility of determining organic nitrogen by direct nesslerization of the ammonia thus formed.



*Columbus method.*<sup>12</sup>—Following lines suggested by the English workers, a method for the direct nesslerization of the neutralized digestate of the Kjeldahl process was developed at the Columbus Sewage Testing Station. By applying the principles of water softening, there were eliminated the calcium and magnesium, whose interference has long been the cause of the turbidity besetting former attempts at direct nesslerization. The details of the method are given in another paper, but, in brief, the neutralized digestate is first treated with an excess of alkali, sodium carbonate is then added to precipitate the calcium, and under these conditions complete softening of the digestate results, allowing the clear yellow color of the mercury ammonium iodide to develop in a normal manner upon the subsequent nesslerization of a suitable portion of the clarified digestate. Potassium permanganate is omitted on the ground that manganous salts might escape oxidation during the preparatory treatment of the digestate, whereby turbid tubes would result on nesslerization, as experiments to cover this point have clearly indicated.

#### THE NECESSITY FOR PRELIMINARY REDUCTION OF NITRATES IN THE KJELDAHL PROCESS.

Some published literature is available on the effect of nitrate nitrogen upon the results of the Kjeldahl process. Rideal<sup>4</sup> describes experiments wherein certain amounts of nitrate were added to sewages to the extent of 68 and 136 parts of nitrogen as nitrate per million, respectively. To these sewages the Kjeldahl process was applied, digesting with 2 c.c. and 4 c.c. sulphuric acid, respectively. The results of the experiment are shown in the following table, from which the conclusion was drawn that the large excess of sulphuric acid used in the Kjeldahl process prevents loss of nitrogen by secondary decomposition.

TABLE 1.  
DR. RIDEAL'S EXPERIMENTS AS TO THE EFFECT OF NITRATES UPON THE RESULTS OF THE KJELDAHL PROCESS.

C.C. SULPHURIC ACID ADDED	TOTAL NITROGEN BY KJELDAHL— PARTS PER MILLION		
	Parts per Million added Nitrogen		Nitrate
	0.0	68	136
2.....	425	350	375
4.....	410	375	390

NOTE.—Sample strongly urinous, contained 350 parts per million nitrogen as free ammonia.

Phelps, in a study of the Kjeldahl process, has published some convincing data with regard to the effect of nitrates. He added nitrate and nitrite nitrogen in varying amounts up to 40 parts nitrate

and 20 parts nitrite per million, respectively, to samples of crude sewage, septic sewage, coarse-grain filter effluents, and sand filter effluents, and determined the unoxidized nitrogen in the several samples before and after treatment. The results showed conclusively that no practical interference need be looked for in the examination by the Kjeldahl process of samples containing as high as 60 parts nitrified nitrogen per million.

The writers have also made experiments upon this point, determining by the direct Kjeldahl process the unoxidized nitrogen in samples of a settled sewage to which nitrate nitrogen was added up to 30 parts per million. From the results given below it is evident that the effect of nitrate is practically negligible.

TABLE 2.  
EFFECT OF NITRATES UPON THE RESULTS OF THE KJELDAHL PROCESS.  
(Average of Duplicate Determinations.)

PARTS PER MILLION NI- TRATE NITRO- GEN ADDED	NITROGEN—PARTS PER MILLION		
	Total	As Free Ammonia	Organic
0.....	15.4	6.4	9.0
10.....	15.4	6.4	9.0
20.....	15.0	6.4	8.6
30.....	14.6	6.4	8.2

NOTE.—0.1 difference on standard is equivalent to 0.4 part per million for 50 c.c. sample.

#### RELATIVE SUPERIORITY OF THE KJELDAHL PROCESS OVER THE ALBUMINOID AMMONIA PROCESS.

The albuminoid ammonia process has been in general use for many years in this country and in England, but only in recent years has been supplanted in the larger laboratories by the Kjeldahl process. The former process has, as we all know, long been recognized as yielding uncertain results, which represent only a portion of the total organic nitrogen obtainable by the use of the Kjeldahl process. The variability of the ratio between the albuminoid ammonia and the Kjeldahl values was well illustrated by the comparative serial analysis made during the staling of a sample of fresh sewage, as reported by Fuller.<sup>13</sup> In this case the ratio varied from 20 per cent to 50 per cent, increasing as the septicity of the sewage increased.

The uncertainties of the albuminoid ammonia results have been shown by a number of observers.<sup>14 15 16 17 18 19 20</sup>

TABLE 3.  
COMPARATIVE DATA ILLUSTRATIVE OF THE VARIABILITY OF THE RELATION WHICH ALBUMINOID  
NITROGEN RESULTS BEAR TO KJELDAHL NITROGEN.

CRUDE SEWAGE.				
LOCALITY	OBSERVER	NITROGEN PARTS PER MILLION		PERCENTAGE WHICH ALBU- MINOID NITROGEN IS OF NITROGEN BY THE KJELDAHL PROCESS
		As Albuminoid Ammonia	Kjeldahl	
Lawrence, Mass.....	Clark	3.0	7.8	39
Hopedale, Mass.....	"	2.2	6.0	37
Leicester, Mass.....	"	3.3	7.2	46
Manchester, Eng.....	Fowler	4.2	10.0	22
Manchester, Eng.....	"	4.8	15.5	31
Belfast, Eng.....	Letts	8.9	15.3	58
Saltley, Eng.....	Watson	15.6	39.1	40
Rhea, Eng.....	"	15.2	48.3	21
Hocklay, Eng.....	"	16.7	59.7	28
Aston, Eng.....	"	19.5	92.8	21
Columbus, Ohio*.....	Johnson and Kimberly	3.5	7.3	48

\*Average of 22 analyses.

NOTES.—Massachusetts data compiled from Report Massachusetts State Board of Health, 1903. English data, from Manchester Reports and Royal Commission Reports, 1902, and Report of Birmingham, Rhea, and Tame Drainage Board, 1903

TABLE 4.  
COMPARATIVE DATA ILLUSTRATIVE OF THE VARIABILITY OF THE RELATION WHICH ALBUMINOID  
NITROGEN RESULTS BEAR TO KJELDAHL NITROGEN.

SEPTIC SEWAGE.				
LOCALITY	OBSERVER	NITROGEN. PARTS PER MILLION		PERCENTAGE WHICH ALBU- MINOID NITROGEN IS OF NITROGEN BY THE KJELDAHL PROCESS
		As Albuminoid Ammonia	Kjeldahl	
Lawrence, Mass.....	Clark	3.0	7.8	39
Hopedale, Mass.....	"	2.2	6.0	37
Leicester, Mass.....	"	3.3	7.2	46
Manchester, Eng.....	Fowler	4.2	10.0	22
Manchester, Eng.....	"	4.8	15.5	31
Columbus, Ohio.....	Johnson and Kimberly	3.6	6.4	56

SEWAGE SLUDGE.				
Brockton, Mass.....	Clark	56.0	181	31
Lawrence, Mass.....	"	13,000.0	23,700	55
Lawrence, Mass.....	"	390.0	860	45
Lawrence, Mass.....	"	633.0	1,190	56
Lawrence, Mass.....	"	731.0	1,644	44
Lawrence, Mass.....	"	222.0	294	76
Lawrence, Mass.....	"	530.0	943	56

EFFLUENTS OF COARSE-GRAIN SEWAGE FILTERS.				
Columbus, Ohio* } .....	Johnson and Kimberly	1.1	2.1	52
Columbus, Ohio† } .....		1.6	3.8	42

\*Contact filter, average of 10 determinations.

†Sprinkling filter, average of 16 determinations.

From the Massachusetts and the English reports the tables on the preceding page have been prepared, which, while necessarily incomplete, may be said to illustrate the great variability of the ratio existing between the results by the two processes and the inherent cause of the decline of the albuminoid ammonia process.

#### METHODS FOR THE DETERMINATION OF NITROGEN AS FREE AMMONIA.

There are two methods for the determination of nitrogen in sewage in the form of ammonium salts, namely, by distillation with sodium carbonate or magnesia, or by direct nesslerization. The latter method is rapidly coming into general use. A digest of current thought and practice in this regard is as follows:

*German method.*—This method consists in a clarification of the sample with caustic soda. In certain cases zinc acetate is also added to remove hydrogen sulphide. Following this preparatory treatment, a suitable portion of the clear supernatant liquid is removed, diluted to 50 c.c., and nesslerized. Phelps<sup>20</sup> has modified the method by diluting the sample to double its original volume before adding the caustic soda, and by using copper sulphate and lead acetate as coagulants.

*English method.*—Rideal<sup>4</sup> and McGowan<sup>5</sup> describe a method for direct nesslerization. Rideal speaks highly of the process, while McGowan says it cannot be used in all cases, since turbid nesslerized tubes result under certain conditions, especially when hard effluents high in calcium are examined. There is an apparent lack of harmonious opinion in England regarding the feasibility of determining nitrogen as free ammonia by a direct process.

From our standpoint the cause of the complications referred to by McGowan are apparently due to the lack of sufficient carbonic acid ions to precipitate the incrusting calcium. In cases where a sewage high in sulphates is examined, in the absence of alkalinity, the technique should include the addition of sodium carbonates as well as caustic soda.

*Columbus method.*—An alternative method, applicable when the sewage contains high magnesium, consists in treating the sample with caustic soda in sufficient amount to precipitate the magnesium and to soften the sewage. A precipitate of magnesium hydrate and calcium carbonate results, whereby there is effected a complete clarification of the sewage. This modification is embodied in the Report of the Committee on Standard Methods.<sup>1</sup> For the typical hard sewage of Columbus, the presence of a lead salt was not required for the removal of sulphureted hydrogen. For sewage low in magnesium the method may be made applicable by the addition of a magnesium salt as a coagulant.

#### RELATIVE RESULTS BY THE DIRECT AND THE DISTILLATION METHODS.

The advantages attached to the direct methods of determining nitrogen in sewage in the form of ammonium salts refer to the fact that by distillation there is obtained a certain amount of ammonia, split off from partially reduced nitrogenous organic compounds on



the border line between ammonium salts and crude organic matter. Further, as has been shown by Phelps,<sup>20</sup> distillation with sodium carbonate causes the development of ammonia from a number of nitrogenous substances. Under such circumstances a direct process should give somewhat lower results, representative of true ammonium compounds alone. As pointed out by Schmidtman and Günther,<sup>8</sup> the direct processes yield results now higher, now lower, than the distillation process, but generally, according to the experience of the writers, averaging about 90 per cent of those obtained by distillation. In the following table are shown the results obtained by the writers upon this point.

TABLE 5.  
COMPARISON OF RESULTS BY THE DIRECT AND DISTILLATION METHODS FOR THE DETERMINATION OF NITROGEN AS FREE AMMONIA.

CHARACTER OF SAMPLES	NUMBER OF SAMPLES AVERAGED	NITROGEN AS FREE AMMONIA PARTS PER MILLION		PERCENTAGE WHICH DIRECT RESULTS WERE OF DISTILLED RESULTS
		Direct	Distilled	
Crude sewage.....	32	13.1	15.1	87
Settled sewage.....	33	13.2	14.8	90
Septic sewage.....	27	12.8	14.0	90
Strained sewage.....	13	14.2	15.0	90
Effluent of coarse-grain filter....	39	10.6	11.8	90
Effluent of sand filter.....	49	2.23	2.5	90

#### THE DETERMINATION OF OXYGEN CONSUMED.

No method has undergone greater modification than that for the determination of the oxygen consumed by, or absorbed from, potassium permanganate.

*Standard method.*—Following the practice of Palmer,<sup>21</sup> the Committee has recommended for use in newly established laboratories a 30 minute contact with acid permanganate in flasks immersed to the neck in a boiling water-bath. This period of contact and this technique were advised for the reason that a fairly complete oxidation is effected thereby, with a minimization of the personal errors incidental to the other modifications.

*Lawrence method.*—The practice at the Lawrence Experiment Station<sup>2</sup> is to boil for two minutes with an excess of permanganate, titrating back with oxalic acid.

*Boston method.*—This method<sup>22</sup> is similar to the Lawrence method except that the period of boiling is five minutes. In both these methods the permanganate is added to the hot solution.

*German method.*—In Germany<sup>7</sup> the period of boiling is uniformly 10 minutes, and the determination is usually made upon the *filtered* solution.

*English methods.*—In England the boiling methods are practiced but little, as considerable error is believed to be introduced through the decomposition of the per-

manganate itself at the temperature of boiling water. The English methods use the "thiosulphate-iodine" reaction for final titration, since oxalic acid is obviously unsuitable in the cold. The many modifications of the oxygen consumed test in current practice in England are as follows:

*The "at once" test.*<sup>23</sup>—In this method, carried out at room temperature, the excess of permanganate is removed within 30 seconds after its application by the addition of potassium iodine.

*The "three-minute" test.*—In this method<sup>24</sup> the permanganate is allowed a contact of three minutes at a temperature of 80° F.

*Other tests.*—Periods of 15 minutes,<sup>25</sup> two and a half hours,<sup>4</sup> and four hours, at 80° F.,<sup>3</sup> are in use in England. Of these, the 15 minute and four hour periods are the more generally practiced. These modifications are said to serve the purpose of differentiating the putrescible matter. As stated by Rideal,<sup>4</sup> at the Manchester Meeting of the Society of Chemical Industry in 1898, the several oxygen consumed tests were said to possess the following characteristics:

1. "The three minute test showed (nitrites, ferrous salts, sulphureted hydrogen and) putrefying matter decomposing permanganate at once with acid."

2. "The difference between three and 15 minutes showed matter readily putrefying and rapidly decomposing acid permanganate."

3. "The difference between 15 minutes and four hours gives matter capable of putrefying, though slow to decompose."

Rideal<sup>4</sup> obtains what he calls a final figure by subjecting the sample to the action of permanganate for two and a half hours at a temperature of 80° C.

#### COMPARISON OF RESULTS BY DIFFERENT METHODS.

These different methods for determining the oxygen consumed or absorbed from acid permanganate bear a fairly definite relation to one another, as is illustrated by the following table:

TABLE 6.  
COMPARISON OF RESULTS BY DIFFERENT METHODS.

SOURCE OF METHOD	TIME OF CONTACT (Minutes)	TEMPERATURE (°C.)	RELATIVE RESULTS TO 5 MINUTE BOIL—AUTHORITY		
			Columbus	Fuller	Kinnicutt
England.....	3	26.7	0.20	0.20	....
England.....	15	26.7	0.33	0.35	0.30
England.....	240	26.7	0.56	0.60	0.48
Palmer.....	30	96.0	3.14	....	....
"Absolute".....	240	100.0	4.73	4.00	....
Lawrence.....	2	100.0	0.74	0.65	0.65
Boston.....	5	100.0	1.00	1.00	1.00
Germany.....	10	100.0	1.18	1.25	1.52

#### LOSS ON IGNITION.

The ignition of the residue remaining upon evaporation of a measured portion of sewage has been practiced for a good many years. The loss in weight sustained is roughly indicative of the total

amount of organic matters present. As is well known, many other substances besides organic matter are volatilized during the ignition, so that the test does not strictly indicate the organic content of the sample. Notwithstanding the limitations of the method, the information afforded thereby is of considerable value in sewage work, as it is the only test whereby even an approximation of the total amount of organic matters present. Relative to questions of the disposal of sludge deposits, the data afforded have a particular field of usefulness.

*Limitations of the process.*—In brief terms, the loss in weight upon ignition includes all organic matters, a certain amount of carbonic acid and combined water split off from the carbonates of the alkaline earth metals, especially magnesium, and further, under certain conditions, a certain loss due to the deoxygenation of mineral salts in cases where the temperature of ignition has been too high. Chlorides are also a factor, when present in considerable amounts.

The factors of error have been corrected to a certain extent by the refinement of the technique of the method in its more recent development. At the same time, as is well known, the variable character of these complications cannot always be allowed for in an entirely satisfactory manner. Even under these conditions, the process is of considerable value, since it throws light upon those constituents regarding whose true nature, amount, and precise composition the present status of analytical methods yields but sparse information.

*German method of determining loss on ignition.*—During the tests made at Cologne, Germany, in 1904,<sup>8</sup> loss on ignition data was obtained by a technique involving repeated partial ignitions at a very low temperature (moving the dish back and forth over a low flame), preceded by the application to the residue of distilled water and subsequent evaporation. From four to six ignitions were usually made; following the final heating, the residue was treated with a few drops of ammonium carbonate solution to convert to carbonate any alkaline earth oxides. With this technique, a very complete combustion of the organic matter was effected, with a minimum loss of volatile mineral matter. This method appears to be a distinct improvement over the technique employed in this country, and, as brought out in another paper,<sup>26</sup> the experience of the writers indicates that a

re-application of distilled water to ignitions made in the radiator following a second ignition might with advantage be included in the standard procedure, as the combustion of the organic matter would thereby be more complete and the results of the process more reliable.

*Greater relative value of volatile suspended matter data.*—Loss on ignition is of course not a test of differentiation. Although the data obtained by ignition of the residue from the evaporation of a measured amount of sewage are to be regarded only as approximations of the constituents they are intended to represent, it is possible, by the use of an entirely different process, to obtain precise information regarding that portion of the suspended matters in sewage which is of a volatile character. To effect this, the suspended matters are separated from the dissolved matters by filtration through asbestos in a Gooch crucible.<sup>26</sup> In the absence of the complicating mineral matters, the volatile suspended matters may be determined by an ignition at a temperature sufficiently high to effect a complete destruction of all organic matters. In problems relating to the preparatory treatment of sewage, these data are of great value, since they indicate to a certain degree the relative composition of the deposits of sludge which are incidental to these processes.

#### CONCLUSIONS.

Casting up the evidence, it seems apparent that more satisfactory practical results may be obtained by the Kjeldahl process than by the use of the albuminoid ammonia process, the results of which are such uncertain percentages of the total organic nitrogen present in sewage and in effluents.

The direct process for the estimation of the ammonia formed in the Kjeldahl method seems practical and reliable above 0.5 part per million organic nitrogen. Below this amount the distillation method of Palmer is available.

The direct determination of nitrogen as free ammonia is safe and accurate; either modification of the process may be used according to local hardness conditions.

Although governed largely by local conditions, the oxygen consumed test, with its many modifications, is to be considered of great value in the examination of crude and purified sewages.



Regarding the loss on ignition, the indications are that the total and volatile suspended matters, as determined directly by the Gooch process, are more reliable and more satisfactory data for sewage problems than either the total volatile matters in the sewage or effluent or the indirectly determined total and volatile suspended matters. The practical advantages of the direct Gooch process render the method worthy of serious consideration, especially in problems wherein suspended matter is an important factor.

None of the current methods for the determination of organic matter distinguish between stable and unstable organic matter. Only by resorting to one of the various methods for the determination of putrescibility can information be obtained regarding the putrescible and non-putrescible matter in partially purified sewage, and even under these conditions there is indicated only the relative predominance of putrescible and non-putrescible substances. It seems to be in this direction that the need is most urgent for methods applicable to the determination of the organic matter in crude and purified sewages.

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## A METHOD FOR THE DIRECT DETERMINATION OF ORGANIC NITROGEN BY THE KJELDAHL PROCESS.

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ONE of the most conspicuous recent advances made in sewage analysis in this country may be said to be the development of a direct process for the determination of the nitrogen as ammonia.<sup>1</sup> The greater simplicity of the technique, the greater economy of time effected by the elimination of distillation, and the relatively greater accuracy of the process are salient advantages of the direct method which surely cannot fail to be of moment in the routine work of sewage laboratories.

To be able to apply a direct process to the determination of the ammonia formed in the determination of total organic nitrogen by the Kjeldahl process seemed the next step toward greater simplicity and rapidity of execution. During a considerable portion of the work at Columbus a direct process was in daily use for the determination of the nitrogen as ammonia, and a more intimate knowledge was gained of local conditions as to the disturbing factor of turbidity, to which, as we all know, has been chiefly due the indifferent success attending earlier attempts at the direct nesslerization of the acid digestate of the Kjeldahl process. Under such circumstances it was thought that a direct method for determining nitrogen by the Kjeldahl process might be successfully developed.

Working along lines by which a direct process for the determination of the nitrogen as free ammonia was successful under such unusually hard water conditions as prevailed at Columbus, a method was finally obtained by which the disturbing turbidity was completely eliminated. It is our purpose to discuss the several factors which have to do with the turbidity occurring in nesslerized tubes, and further to describe a direct method for determination of nitrogen by the Kjeldahl process, found applicable under Columbus condi-

tions, together with certain suggestions whereby it appears that the direct method may be made of quite extended applicability.

#### METHODS PREVIOUSLY PROPOSED.

A review of the literature upon the question of the direct nesslerization of the acid digestate of the Kjeldahl process indicates that the matter has received but little attention. So far as we are aware, the method described by Rideal,<sup>2</sup> a similar one used to some extent by McGowan,<sup>3</sup> and the modifications suggested by Phelps,<sup>1</sup> are the only instances on record in which the feasibility has been suggested of the elimination of distillation in Kjeldahl nitrogen determinations. Rideal states that the direct determination of the organic nitrogen has been practiced to some extent in England. The method as given by him is as follows:

*"Kjeldahl.*—10 c.c. of a sewage, or say, 100 c.c. of an effluent plus 4 c.c. of pure sulphuric acid, are heated in a pear-shaped hard glass flask in a slanting position until the liquid becomes colorless. When about 2 or 3 c.c. remain, the flask is cooled and is washed out with small quantities of ammonia-free water into a 100 c.c. measure, until the volume of the liquid reaches about 40 c.c. An excess, i. e., about 25 c.c., of soda solution (25 per cent) free from ammonia is now added, when a flocculent precipitate is thrown down. After cooling, the liquid is made up to 100 c.c., transferred to a clean and dry stoppered bottle, and shaken at intervals until the flocculi—which at first float entangled with air bubbles—subside. A suitable fraction of the clear liquid is then pipetted into a Nessler glass, diluted to 50 c.c., and nesslerized. *This gives the total unoxidized nitrogen in terms of ammonia.*"

This method was used by McGowan,<sup>3</sup> modified only by the occasional addition of potassium oxalate solution to the alkaline digestate to precipitate the calcium, and by the proviso that the solution should be "rendered *just* alkaline with purified potash." The method is criticised by McGowan on the ground that turbidity is oftentimes noted, even when the calcium has been removed by the addition of oxalate solution, and that considerable entrainment of ammonia results from the precipitate formed upon the neutralization of the digestate. His chief objection to the process seems to be that where considerable amounts of nitrified nitrogen are present, all of the unoxidized nitrogen is not recovered, since, during the last stages of the digestion, a portion of this nitrogen is oxidized by the nitric acid present and is probably lost in the form of lower oxides of nitrogen. For this reason the English practice in regard to the Kjeldahl method for effluents includes a reduction of all nitrified



nitrogen by the use of zinc and sulphuric acid. This portion of the procedure is stated by English workers to require five days for its completion; for otherwise, as their experience indicates, an appreciable loss of nitrogen will ensue. The discussion is concluded with the statement that although the direct method—with no reduction of nitrate—has been found at times to yield very satisfactory results in the analysis of very pure effluents, and frequently in others containing as high as 5.0 parts per million nitrogen as nitrate, yet, for general work upon sewage and sewage effluents, experience seems to be against the method, since no preliminary reduction of nitrate is included in the technique.

In this country, Phelps has outlined a method which he used with somewhat indifferent success, owing, as he states, to certain factors associated with the turbidity of the nesslerized tubes, which as yet are not clearly understood. The method employed by him consisted in making up the digestate to 250 c.c., removing 2 c.c., neutralizing, making up to 50 c.c., and directly nesslerizing.

In the nitrogen work at the Columbus Sewage Testing Station a study was made of the feasibility of adopting a direct method for the determination of the ammonia formed in the Kjeldahl process, along the lines suggested by the studies of previous workers to which we have just referred. With some modifications, the methods described by Rideal and McGowan have been found to yield satisfactory results when applied to the sewage and effluents under study at Columbus. Those changes were the results of an extended inquiry into the causes of the disturbing turbidity which, as we all know, has been the chief drawback to the applicability of the direct process. The results of these studies have been incorporated in the technique of the method as given below, and it is felt that the method, when modified along lines to be suggested in order to meet different conditions, gives promise of a fairly wide field of applicability in the analysis of sewage and sewage effluents.

#### FACTORS ASSOCIATED WITH THE TURBIDITY PRODUCED BY DIRECT NESSLERIZATION.

The cause of the disturbing turbidity which appears upon the addition of Nessler's reagent to solutions containing other salts as well as ammonium salts, has been chiefly attributed to the presence

therein of calcium salts. Considering for the moment such conditions as would obtain in direct nesslerization, without treatment with caustic soda, of an aliquot portion of a hard sewage containing the bicarbonates of calcium and of magnesium, respectively, and, in addition, both these metals as incrustants, it follows from water-softening experience that the addition of the caustic Nessler's reagent will entail a precipitation of a portion of the calcium as carbonate, and the whole of the magnesium as hydrate, whereby cloudiness would result. Such seems to be one of the explanations of the failure of attempts to obtain clear tubes when small amounts of a hard sewage, or sewage containing but moderate amounts of the alkaline earth metals, diluted to the usual 50 c.c., have been directly nesslerized, or when, as in Phelps's method, a small portion of the acid digestate is neutralized and nesslerized without preparatory treatment for the removal of the magnesium, and in certain cases of calcium as well.

The method as advised by the Committee on Standard Methods for the determination of the nitrogen as free ammonia by direct<sup>4</sup> nesslerization, appears to admit of adoption under a wide range of conditions with respect to the mineral constituents of the sewage, for the reason that the preparatory treatment of the sample with caustic alkali effects a complete softening of a hard sewage, removing to a sufficient degree the carbonate of calcium, all of the magnesium, and a variable proportion of calcium sulphate, depending upon the relative amount of half-bound carbonic acid present. The development of a successful method for the direct nesslerization of ammonia has clearly resulted from the use of a preparatory treatment which softens the sewage materially, thereby removing before nesslerization those substances which, by precipitating in the nesslerized tubes, caused the turbidity so often observed.

Coming now to the premises of the paper, the thought arises as to wherein the conditions differ from those obtaining in the successful direct determination in hard sewages of the nitrogen as free ammonia. As is well known, under certain conditions the metals of the alkaline earth group, when present in a nesslerized tube, obscure by precipitation the clear yellow of the mercurio-ammonium compound, and thus the question of the turbidity of the neutralized and nesslerized

lerized acid digestate would appear to revert to the interference of calcium and magnesium salts, and, further, possibly to the presence of sodium sulphate from the neutralization of the sulphuric acid.

A little thought will suggest that, irrespective of the nature of the acid radicals to which the calcium and the magnesium are united in the sewage, they are in combination, after digestion, with sulphuric acid as sulphates. The neutralization of the acid digestate with a sufficient excess of alkali removes the magnesium as in the direct ammonia procedure, so that, so far as these metals are concerned, the question of turbidity would appear to refer to the presence, of calcium as sulphate, which is not removed upon the neutralization of the acid digestate with carbonate-free caustic soda. We have found by experiment that calcium as sulphate may be present in a nesslerized tube without causing precipitation, to the extent of 50 parts per million of calcium (Ca). Above this amount, up to the limit of the solubility at ordinary temperatures of calcium as sulphate of 100 parts per million of calcium (Ca), turbidity will be noted. Under ordinary conditions, calcium in this form is probably not a factor; but to avoid possible complications, under conditions to be referred to later, it would seem advisable to add to the digestate a certain amount of carbonate of soda, for the removal of the calcium, imitating conditions obtaining in most cases in the direct nesslerization of the nitrogen as ammonia. To study the effect of the presence of sodium salts, a blank digestate was neutralized and carried through the usual procedure, as given below, and in no case did any turbidity appear to be traceable to this source.

Taking into account each of the factors just discussed, efforts to obtain clear tubes continued to be indifferently successful, until finally it developed that the character of the caustic soda used for neutralization was related to the intensity of the turbidity of the nesslerized tubes. The so-called C. P. stick caustic soda is far from pure, as is well known, and it was found that the usual treatment for the removal of ammonia was not sufficient to prevent a precipitation in nesslerized tubes containing free alkali. To remove ferrous iron, organic matter, or any chance reducing agent to any of which the turbidity would appear to be due, caustic soda was prepared, by oxidation with either sodium peroxide or permanganate, as

described below. With caustic solutions prepared in this manner, fairly satisfactory results were obtained.

Up to this time the method described by Palmer<sup>5</sup> had been used, wherein permanganate is added to the digestion when it is judged to be complete. In reviewing possible causes of the disturbing turbidity, the thought suggested itself that the presence of manganous sulphate, even in small amounts, would tend to produce turbidity, because of the ease with which the salt is oxidized and precipitated in alkaline solutions. Experiments with solutions containing manganous sulphate (formed when permanganate is used) showed conclusively, as was expected, that turbid tubes would result upon nesslerization. As the use of permanganate is of uncertain value as an aid in the oxidation by sulphuric acid of the substances found in ordinary sewage, following the practice of Rideal,<sup>2</sup> the addition of permanganate at the end of the digestion was omitted from the procedure as finally adopted, on the ground that a certain amount of manganous sulphate might escape oxidation during the preparation of the digestate for nesslerization, whereby turbid tubes would result.

By the use of a caustic soda solution free from oxidizable organic matter, clear tubes were finally obtained, provided that a sufficient excess of caustic was added to precipitate completely the magnesium, and provided that sodium carbonate was added to remove the calcium.

Considerable literature is available upon the use of Nessler's reagent, and from the work of Nessler,<sup>6</sup> Miller,<sup>7</sup> Chapman,<sup>8</sup> Frankland and Armstrong,<sup>9</sup> Trommsdorff,<sup>10</sup> and Fleck,<sup>11</sup> it appears that both free alkali and the presence of magnesium, or the formation in the tubes of any other precipitate whatsoever, affects the color produced by the reagent. The alkali tends to give abnormally high readings, and the co-precipitation of any other substance, together with the mercury ammonium iodide, completely coagulates the colloidal color produced by Nessler's reagent in pure solutions of ammonium salts. In connection with the question of the direct nesslerization under hard water conditions at least, it is a significant fact that the use of a magnesium salt forms the basis of Fleck's titration method<sup>11</sup> for the determination of ammonia by Nessler's reagent, wherein the mercury ammonium iodide, completely precipitated by magnesium,



is dissolved in sodium thiosulphate and titrated with an ammoniacal solution of silver sulphide.

It is evident that an excess of alkali will remove the magnesium. To determine the limit to which the excess could be carried with safety, different amounts of differently prepared caustic soda solutions, ranging by 10ths to one gram, respectively, were added to Nessler tubes containing 3 c.c. of ammonia standard. The tubes were filled to the mark with ammonia-free water, and were then nesslerized. The colors were read after 15 minutes. It appears from the results in the table below that 0.2 gram of pure caustic (NaOH) may be present in a nesslerized tube without producing turbidity or causing the color to be abnormal. Beyond this amount, turbidity, and finally complete precipitation, will result, as will also be the case when impure caustic is used without an oxidizing treatment.

Above 0.5 gram a heavy precipitate was observed in all cases.

TABLE I.

EFFECT OF THE PRESENCE OF CAUSTIC SODA UPON THE COLOR PRODUCED BY NESSLER REAGENT.

CAUSTIC SODA (NaOH) (Gram)	TREATMENT RECEIVED BY CAUSTIC*							
	NONE				SODIUM PEROXIDE		PERMANGANATE	
	FUSED STICK		BY ALCOHOL					
	Reading	Turbidity	Reading	Turbidity	Reading	Turbidity	Reading	Turbidity
0.0.....	3.0	o	3.0	o	3.0	o	3.0	o
0.1.....	...	str.	3.0	o	3.0	o	3.0	o
0.2.....	...	"	3.0	o	3.0	o	3.0	o
0.3.....	...	"	3.1	o	3.1	o	3.2	o
0.4.....	...	"	3.5	sl.	3.3	v. sl.	3.5	v. sl.
0.5.....	...	"	3.8	str.	3.5	str.	3.6	str.

\*Fused white sticks.

By including in the final details of the method an excess of alkali corresponding to 2 c.c. of 25 per cent caustic soda, or 5.0 gram of the alkali giving in the nesslerized tubes only one-tenth of this amount and adding 0.2 gram of sodium carbonate to the alkali digestate, clear tubes were finally obtained. The method employed takes into account the following factors, which our experience indicates as the controlling causes of turbid tubes in direct nesslerization work:

a) Insufficient preparatory treatment of the sample, whereby there results a precipitation of calcium carbonate or of magnesium hydrate,

and, in general, the formation by the alkali of the Nessler's reagent of any other precipitate whatsoever save the normal colloidal mercury ammonium iodide.

- b) The use of caustic soda containing organic matter.
- c) The presence of a manganous salt from the use of permanganate in the digestion.
- d) The presence in the nesslerized tubes of calcium as sulphate in hard sewages above 50 parts per million, and, further, the neglect to mix thoroughly the solution before nesslerizing, even in cases where the calcium in a tube containing a homogeneous solution would be below the limiting value of 50 parts per million.

#### DETAILS OF THE COLUMBUS METHOD FOR THE DIRECT DETERMINATION OF NITROGEN BY THE KJELDAHL PROCESS.

Although subject to considerable modifications from time to time, to correspond to developments in the study of the turbidity question, the Columbus method for the direct determination of nitrogen by the Kjeldahl process finally assumed the following definite shape:

*Reagents.*—Aside from the preparation of the caustic, the use of a sodium carbonate solution, and the omission of permanganate, the reagents do not differ from those used in the standard Kjeldahl process.<sup>5</sup> Two brands of caustic soda were used, one the ordinary fused white sticks, the other caustic soda from alcohol. Both require a certain amount of oxidation to remove all organic matter. This has been effected either by the addition of about 2 per cent of sodium peroxide to the caustic solution prepared from caustic by alcohol, or, in case the ordinary fused white stick caustic, which is somewhat cheaper than that from alcohol, is used, by the use of potassium permanganate as follows:

Caustic soda solution: Dissolve in a liter of redistilled water 250 grams of fused white stick caustic soda. Heat to boiling and add a strong solution of potassium permanganate, drop by drop, until a pale-green color persists; continue to boil to expel ammonia and to cause a precipitation of the manganic oxide.

If the conditions are right, the caustic solution will assume a coffee-brown color, due to the suspended manganic oxide. In cases where the caustic is desired for immediate use, the solution, when cooled sufficiently, is filtered through asbestos by the aid of a filter pump; the resulting filtrate should be perfectly colorless, and free from suspended matter. Where time permits, the turbid solution may be allowed to stand until the precipitate shall have subsided.

Carbonate of soda solution: Dissolve 100 grams of C. P. anhydrous salt in one liter of ammonia-free distilled water.

*Method.*—Measure out such an amount of the sample as will contain between 0.00050 and 0.00100 gram of nitrogen; that is, an amount which will be equivalent to from 50 to 100 c.c. of the nitrogen ammonia standard; and digest with 5 c.c. of sulphuric acid, until the liquid is colorless or, in the presence of iron, shows a pale-

yellow color which becomes white when the solution has cooled. Rinse the acid digestate into a 50 c.c. flask, allow to cool to room temperature, fill to the mark, and mix thoroughly by inverting the flask at least four times. Place 25 c.c. of this solution in a 100 c.c. flask, preferably glass-stoppered, and add an amount of caustic soda solution not quite sufficient to neutralize the acid completely. Immerse the flask in ice-water to effect a rapid cooling. When cool, add more caustic soda solution from a pipette, drop by drop, until a flocculent precipitate is plainly visible. Add then 2 c.c. of the sodium carbonate solution. Make up to the 100 c.c. mark, stopper the flask, and mix thoroughly. Pour the solution into a clean four-ounce white glass bottle, preferably tall, and allow to stand at least six hours, shaking slightly at intervals in case the flocculi do not tend to settle readily. Pipette 10 c.c. of the completely clear, colorless supernatant solution into a 50 c.c. Nessler tube, make up to the mark with ammonia-free water, and mix thoroughly by inverting the tube. Nesslerize as usual and read after 15 minutes. The nesslerized tubes should show no turbidity. As in all Kjeldahl methods, a reagent blank must be subtracted from each determination. The results are computed as follows:

$$\frac{(N \times 20) - \text{Blank}}{S} \times 1,000,000 \times .0001$$

Where  $N$  = c.c. standard reading

$S$  = c.c. sample digested.

The results are in terms of the *total unoxidized nitrogen* in the sample. The *organic nitrogen* is determined by subtracting from these results the amount of ammoniacal nitrogen determined by a direct process.<sup>12</sup>

#### DISCUSSION OF CERTAIN IMPORTANT FEATURES OF THE METHOD.

There are several points relative to the method, which our experience has shown to be very essential to the attainment of satisfactory results.

*Turbidity.*—To avoid cloudy tubes a period of subsidence of at least six hours is necessary both to insure a clear supernatant solution and also to effect a complete precipitation of the slowly forming calcium carbonate. Further, sufficient carbonate of soda must be present to precipitate the calcium, and a sufficient excess of purified caustic soda solution is necessary for the complete removal of magnesium. An excess of 0.05 gram caustic soda (NaOH) was found to be sufficient. Since, as pointed out above, 0.2 gram of free caustic alkali does not appear to interfere with Nessler's reagent, 0.05 gram of alkali, the amount in each tube, may be safely used, thus insuring a complete precipitation of magnesium.

*Neutralization.*—The neutralization has been completed in two stages, cooling the solution before alkalinity obtains to guard against any possible loss of ammonia. Ice-water has been found convenient as a cooling agent.

*Apparatus and technique.*—In the technique of the method, ordinary graduated volumetric flasks have been used for making the necessary measurements, and for mixing the several solutions. For general practice it would certainly be advantageous to use glass-stoppered 50 and 100 c.c. flasks, respectively, in order that mixing may be carried on without contamination and without injury to the hands.

The containers in which the neutralized digestates are allowed to settle should preferably be tall, in order to facilitate the subsequent pipetting of the supernatant solution. During subsidence, the bottles should be protected from dust and carbonic acid, and to this end it is suggested that glass-stoppered bottles of about 120 c.c. capacity, contained in suitable racks, could be advantageously used to protect the determination prior to the completion of the process.

#### RELATIVE RESULTS FROM THE DIRECT AND DISTILLATION PROCESS, RESPECTIVELY.

In the work at Columbus, before substituting the direct method for the distillation process in current use, a sufficient number of comparative analyses were made to insure that the results should be strictly comparable to those of the older method. At the same time, it was thought that the simpler technique of the direct method and the elimination of all factors of error associated with the distillation might be the means of giving somewhat higher results. Representative results are shown in the table below, from which it appears that the results by the direct process are substantially the same as those obtained by the distillation of the neutralized digestates. The differences observed in the results by the two methods are so slight as to be outweighed, from a practical standpoint, by the very great saving of time effected by the use of the direct process.

#### ENTRAINMENT OF AMMONIA.

One of the objections to the direct process raised by McGowan<sup>3</sup> refers to the possible loss of ammonia through absorption by the precipitate formed when the acid solution of the digestate is neutralized, or when, as in the direct determination of the nitrogen,<sup>4</sup> as free ammonia a more or less heavy precipitate forms upon the addition of alkali. McGowan made experiments to illustrate this by nesslerizing a



TABLE 2.  
REPRESENTATIVE ORGANIC NITROGEN RESULTS BY KJELDAHL—COMPARISON OF THE DIRECT AND  
DISTILLED METHODS.

Parts per Million—Nitrogen.

SOURCE OF SAMPLE	ORGANIC	
	Direct	Distilled
Crude sewage.....	10.1	9.7
	4.5	4.5
	9.0	8.6
	10.3	9.9
Settled sewage.....	4.9	4.7
	10.7	10.3
	5.1	5.1
	13.9	14.0
Septic sewage.....	5.4	5.4
	4.7	5.2
	5.4	5.1
	7.0	7.4
Coarse-grain filter effluent.....	4.7	5.2
	3.9	3.9
	2.2	2.2
	1.8	1.8
Ditto settled.....	3.5	3.7
	1.8	1.9
	2.1	2.2
	3.6	3.0
Sand filter effluents.....	0.67	0.66
	0.37	0.44
	0.53	0.50
	0.65	0.54

portion of the clear liquid in the direct Kjeldahl process, then shaking up the precipitate in the remaining liquid, subjecting the turbid solution to distillation, and determining the amount of ammonia in an aliquot portion of the distillate. He shows that an appreciable entrainment of ammonia results, but adds that a stricter comparison would have been effected had both the clear and the turbid liquid been subjected to distillation. Phelps,<sup>1</sup> in a recent paper upon the determination of ammonia in sewage, alludes to the entrainment factor, which he substantially eliminates by diluting the sample in which it is desired to determine the nitrogen as free ammonia before the addition of alkali.

*Entrainment experiments.*—To study this point under Columbus conditions, a portion of the clear supernatant solutions from the nitrogen as free ammonia and from the total nitrogen determinations, respectively, were nesslerized as usual, following which 40 c.c. of the alkaline solutions, containing probably all of the precipitate, were respectively dissolved in small amounts of sulphuric acid. The solutions were then diluted to 100 c.c., and the calcium and magnesium salts again precipitated by the addition of caustic soda. Aliquot portions of the clear supernatant solutions, corresponding in amounts of ammonia to those in the original determinations, were then diluted to 50 c.c., and the ammonia determined as usual. If the entrainment factor were one of appreciable moment, it was thought that the ammonia reading in the tubes from the

second precipitation would be correspondingly higher than those obtained in the original determination, since the second digestion contained practically all the precipitate formed in the initial precipitation. Since practically the same readings were obtained in both cases and in both of the direct methods, the indications are that the entrainment factor is not of sufficient moment, from a practical standpoint, to render the results of direct processes unreliable. Experimental results upon the absorption factor are shown in the following table:

TABLE 3.  
EFFECT OF ENTRAINMENT UPON RESULTS OF DIRECT NESSLERIZATION.  
Nitrogen as Free Ammonia—Direct Kjeldahl.

NUMBER	STANDARD READING			
	A	B	A	B
1.....	4.0	3.9	2.4	2.4
2.....	3.5	3.5	2.9	2.9
3.....	3.5	3.5	2.2	2.2
4.....	3.1	3.1	2.9	2.7

NOTE.—A=Supernatant from first precipitation; B=ditto from second precipitation.

#### DISCUSSION OF THE GENERAL APPLICABILITY OF THE DIRECT PROCESS IN SEWAGE WORK.

In considering the method for the direct determination of the total unoxidized nitrogen by the Kjeldahl process, which has been found applicable for Columbus conditions, where unusually large amounts of calcium and magnesium are encountered, with respect to its applicability for sewages common to New England and to other places where soft water conditions are as a rule more in evidence, Columbus experience leads us to believe that the method can be successfully used. Since a digestate must be freed from the alkaline earth metals before a direct treatment with Nessler's reagent is attempted, it would seem, in the absence of the complicating salts of hard sewages, that no difficulty would be experienced in obtaining clear tubes.

In this connection it may be stated that the direct process has been applied in the Columbus work to the determination of total nitrogen in bacterial culture media work, in connection with studies of denitrification such as have recently been brought forward at the Lawrence Experiment Station.<sup>13</sup> Excellent results were obtained by the direct nesslerization of aliquot portions of neutralized digestates, in which the Gunning modification<sup>14</sup> of the Kjeldahl process was employed.

For soft-water sewages the method appears to be applicable. Even for harder sewages than a maximum of about 600 parts per million as calcium carbonate, encountered under Columbus conditions, it is thought that the calcium in solution as sulphate would be reduced below 50 parts per million in the nesslerized tubes by the addition of sodium carbonate, and in view of the fact that calcium as sulphate is not soluble over 100 parts per million<sup>15</sup> in pure water and probably much less so in solutions of sodium sulphate. In the case of a very hard sewage, or where a moderately hard sewage low in nitrogen is examined, as we have often noted, considerable calcium sulphate separates out when the digestate is diluted to a volume of 50 c.c. In all cases, therefore, the added sodium carbonate should be sufficient to remove all of the interfering calcium, even when present to the limit of its solubility.

#### APPLICATION TO WATER ANALYSIS.

Outside of the field of sewage analysis is the question of the feasibility of using a direct process in the sanitary analysis of water, in those cases where the total organic nitrogen is determined by the Kjeldahl method. No literature upon this matter has come to our attention.

By modifying the technique to include the neutralization of the entire digestate, with the addition of the usual excess of caustic soda, by adding a suitable amount of sodium carbonate, and by nesslerizing up to 50 c.c. of the alkaline digestate, it appears highly probable that a direct process might be used in water work. We would be inclined to place the limit of total nitrogen obtainable with the direct process at 0.1 part per million.

For such small amounts of total unoxidized nitrogen, complete softening is absolutely essential to the success of direct nesslerization. Moreover, since the direct process for the determination of the nitrogen as free ammonia cannot be applied to the determination of such small amounts as usually accompany low total unoxidized nitrogen values, the advantages of a direct process are not so apparent, especially since distillation processes give small amounts of ammonia with such relatively greater accuracy than can be obtained by a direct process, in which errors of multiplication are always an accompanying feature. It is felt, however, that the direct process may have a certain field of

usefulness for fairly polluted waters, and it is hoped that the suggestions made herein may be of some value to other workers, to whom the opportunity may be given for studying the further development of the direct process along lines which we have not been able to pursue under the hardness conditions obtaining at Columbus.

In conclusion, we desire to express our thanks to Mr. Julian Griggs, chief engineer of the Board of Public Service, Columbus, Ohio, by whose permission we have been enabled to present the results of these analytical studies before the publication of the report of the Testing Station. We wish also to express our appreciation of the valued criticisms of Mr. George W. Fuller, consulting engineer, and the valuable suggestions of Mr. George A. Johnson, engineer in charge of the Testing Station.

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## THE PRACTICAL ADVANTAGES OF THE GOOCH CRUCIBLE IN THE DETERMINATION OF THE TOTAL AND VOLATILE SUSPENDED MATTER IN SEWAGE.

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THE determination of the total and volatile suspended matter in sewage and sewage effluents has recently become of considerable importance, owing to the rapidly increasing number of sewage disposal problems in which conditions necessitate the adoption of filters of coarse-grain material, generally preceded by settling or septic tanks. In determining the efficiency of these latter-day sewage works, and in special investigations incidental to the design of plants of this character, it is highly important that accurate data be obtained upon the amount and character of the suspended matter in the raw sewage. For it is this point which, in a very large measure, controls decisions regarding the character of the preparatory treatment which is best fitted to a given set of conditions, and to which are closely related, also, decisions regarding the character of the finishing process.

In the course of the investigations at the Testing Station, Columbus, Ohio, the question of the removal of the suspended matter in crude sewage under different velocities was made the subject of special study, as will be reported elsewhere, necessitating a large number of determinations of both total and volatile suspended matter. Owing to the pressure of other lines of routine analytical work, and the large amount of expensive apparatus necessary in connection with the usual indirect platinum evaporation method for work of this character, the direct Gooch crucible method, used to advantage by McGowan<sup>1</sup> in sewage work, was substituted in a material measure for the more tedious indirect platinum method. Our experience in this connection indicates that this direct method possesses advantages sufficiently distinct to warrant its serious consideration by other workers

<sup>1</sup>*Fourth Report Royal Com. on Sewage Disp.*, 4. 30, Pt. 5, p. 47.

interested in the determination of total and volatile suspended matter in sewage.

So far as we are able to learn, the application of the Gooch crucible to the determination of the suspended matter in water was first suggested by Thomas and Hall<sup>1</sup> of Philadelphia; in sewage analysis, so far as we are aware, it was developed by McGowan, as described in detail in the *Chemical Report to the Royal Commission on Sewage Disposal*, 1905. The author states, as a result of his experience, that the Gooch crucible may be relied upon to give very accurate data upon suspended matter in sewages, with a minimum expenditure of time. We have carefully studied, under Columbus conditions, the reliability and the general practical advantages of this method for the determination of suspended matter in sewage and sewage effluents, and our results indicate the correctness of the assertions of the English workers.

This paper will be devoted chiefly to a detailed account of our experience with the Gooch crucible in sewage work, referring to the determinations of the volatile, as well as to the total suspended, matter, together with a discussion of the relative merits, from a practical standpoint, of the Gooch crucible and the indirect platinum methods.

#### DETAILS OF THE COLUMBUS METHOD FOR THE DETERMINATION OF THE TOTAL AND VOLATILE SUSPENDED MATTER BY THE GOOCH CRUCIBLE.

*Preparation of asbestos.*—Asbestos adapted for use in the Gooch crucible may be readily prepared from the granular commercial product by digestion on a water bath in strong hydrochloric acid for several hours.<sup>2</sup> By successive decantations with distilled water, the acid is completely removed, leaving the asbestos practically free from iron. High-grade, long fiber asbestos has not been found to yield as good results as the commercial asbestos purified as above described.

*Preparation of mat.*—Prepare a dilute cream of the washed asbestos, which must be free from coarse particles, attach the crucible\* to the filter flask in the usual manner, start the suction, and form a mat about  $\frac{1}{16}$  inch thick upon the bottom of the crucible. After the asbestos has drained completely, apply to the crucible a small quantity of distilled water. If the mat is of the correct thickness, the distilled water will pass through the filter at the rate of about 50 drops per minute. Place the crucible in an oven at 110°–120° C. for 15 minutes; remove and ignite in a radiator for five minutes; cool in a desiccator and weigh. Before each weighing it is expedient to cleanse thoroughly with a soft cloth the outside surfaces of the crucibles. In the absence

<sup>1</sup>*Jour. Am. Chem. Soc.*, 1902, 24, p. 538.

<sup>2</sup>*Fourth Report Royal Com. on Sewage Disp.*, 4, Pt. 5, p. 47.

\*Royal Berlin solid porcelain crucibles were used for this work.

of a platinum radiator, we have found that a four-inch nickel dish, heated to a dull red heat, admirably serves the purpose of this method. During the ignition it has been the practice to allow the crucible to rest directly upon the bottom of the radiator.

*Filtration.*—Use 50 c.c., 100 c.c., or more of the sample, decanting into the crucible as great an amount as possible of the supernatant water before the main portion of the suspended matter is applied thereto; in this way the filtration will be the more rapidly accomplished. Allow the liquid to disappear completely before adding subsequent portions of the sample, also to facilitate filtration. When the filtration is completed, rinse out the flask with about 15 c.c. of distilled water. To guard against imperfect filtration, it is advisable to apply the suction gradually. In case the filtrates are cloudy, they must be refiltered until clear. With a properly prepared mat, our experience indicates that imperfect filtration is of rare occurrence.

*Drying and igniting.*—The crucible is dried at 110°–120° C. for one hour, cooled in a desiccator, and weighed, the increase in weight representing the *total suspended matter* in the sample. To obtain the *volatile suspended matter*, the weighed crucible is ignited in the radiator at a low red heat, for 10 minutes or to constant weight. The completeness of the ignition may be judged by the appearance of the residue. The suspended matter in sewage work is usually black in color, owing to the presence of sulphide of iron. On ignition, as is evident, this is oxidized to ferric oxide, which usually predominates sufficiently to impart to the thoroughly ignited residue, when cool, a reddish-brown color. Our experience indicates that an ignition of 10 minutes is sufficient to effect a complete oxidation of the organic matter present.

*Removal of mat.*—To prepare the crucibles for further use, remove the mat, rinse well in tap water, and finally with distilled water, making sure that the perforations in the bottom of the crucibles are not clogged.

A synopsis of the technique is as follows:

1. Prepare the asbestos mat,  $\frac{1}{8}$  inch thick.
  2. Wash in distilled water.
  3. Dry the crucible at 110°–120° C. for 15 minutes.
  4. Ignite for five minutes.
  5. Weigh. (No. 1.)
  6. Affix crucible to filter flask and start suction pump.
  7. Filter sample, decanting supernatant into crucible.
  8. Wash residue with distilled water.
  9. Dry for one hour at 110°–120° C.
  10. Cool in desiccator.
  11. Weigh. (No. 2.)
  12. Ignite at low red heat in a radiator for 10 minutes.
  13. Cool in desiccator.
  14. Weigh. (No. 3.)
- Weight No. 1=weight of crucible and mat.  
 Weight No. 2=ditto, plus suspended matter.  
 Weight No. 3=same as No. 2, minus volatile matter.

#### RELATIVE RAPIDITY AND ACCURACY OF THE GOOCH CRUCIBLE METHOD.

The determination of the solid matters in sewage and in water, as is well known, requires considerable care and involves a large expen-

diture of time. Where suspended matter results alone are desired, the advantages of a direct method are at once apparent. With the Gooch method the results under routine operation should be available in at least two hours.

That an idea may be had of the accuracy of the Gooch method for the determination of suspended matter in samples wherein the matters in suspension vary widely in amount and character, the following table has been prepared, which contains representative data for a large number of determinations.

TABLE 1.  
REPRESENTATIVE DUPLICATE DETERMINATIONS OF SUSPENDED MATTER BY THE GOOCH CRUCIBLE.

NATURE OF SAMPLE	SUSPENDED MATTER, PARTS PER MILLION			
	Total		Volatile	
Crude sewage.....	183	184	106	109
" " .....	310	314	148	152
" " .....	130	134	95	96
Settled sewage.....	125	128	92	94
" " .....	214	217	103	105
" " .....	266	267	130	128
Effluents of coarse-grain filters.....	144	139	45	44
" " " " " .....	63	67	24	21
" " " " " .....	88	89	26	24
Average.....	169	171	85	87

#### ACCURACY AND RELATIVE ADVANTAGES OF THE GOOCH CRUCIBLE AND THE PLATINUM EVAPORATION METHODS.

*Total suspended matter.*—It is evident that the data obtained from the Gooch method will more correctly represent the actual weight of suspended matter, inasmuch as in all other methods the efficiency of sedimentation and of the filtration is not sufficient to include colloidal matter or substances in a very fine state of subdivision. It would therefore be expected that slightly higher results would be obtained by the Gooch method, were all the other factors associated with the indirect platinum method left out of consideration.

To illustrate the extent to which the colloidal character of the suspended matter affects the accuracy of the usual indirect platinum method, the data in the following table are given. In this table widely different samples of crude sewage were examined by the platinum method in two ways; first, after the usual filtration through paper, and second, after filtration through asbestos, as in the Gooch



process. For the sake of comparison, the Gooch results were also included in the study.

The results show with considerable clearness the extent to which the efficiency of the filtration influences the results by the platinum method. The range of colloidal matter, as indicated from the data in the table below, is from 4 to 22, averaging 12 parts per million. From a practical standpoint it should be borne in mind that the platinum results are of equal value, since the suspended matter which is not included by the evaporation method forms a portion of that amount which cannot be removed by plain subsidence in an economical period of time.

TABLE 2.  
REPRESENTATIVE RESULTS BY PLATINUM AND GOOCH METHODS, SHOWING THE EFFECT OF COLLOIDAL MATTER IN CRUDE SEWAGE.

SAMPLE OF CRUDE SEWAGE	SUSPENDED MATTER, PARTS PER MILLION			APPARENT COLLOIDAL MATTER, PARTS PER MILLION
	By Platinum Method Filtered through		By Gooch Method	
	Paper	Asbestos		
1.....	06	102	00	Max. 22 Min. 4 Average 12
2.....	04	98	05	
3.....	98	114	100	
4.....	72	86	06	
5.....	188	200	103	
6.....	170	102	180	
7.....	362	364	368	
8.....	196	210	223	

In the course of our investigations, a large number of determinations were made, comparing the Gooch and the platinum methods. The direct determination has always given higher results, ranging from 1 to about 33 per cent, and averaging about 13 per cent. As noted above, the completeness of the filtration seems to be one of the principal causes of these differences. The discrepancies are greatest where the suspended matters are low, and especially where they are more or less colloidal in character. Thus, in the table below it may be noted that the settled sprinkling filter effluents give the greatest variation, for in these the suspended matter is usually quite finely divided.

Without detracting from the value of the platinum results, which of course may be made more perfect by filtration through asbestos

or a Berkefeld filter,<sup>1</sup> our experience indicates that the Gooch crucible method is of more practical applicability, owing to the relatively greater facility and speed with which suspended matter data may be obtained. Relative results by the two methods are shown in the following table:

TABLE 3.  
REPRESENTATIVE RELATIVE TOTAL SUSPENDED MATTER RESULTS BY GOOCH AND PLATINUM METHODS.

SOURCE OF SAMPLE	SUSPENDED MATTER, PARTS PER MILLION		PER CENT WHICH GOOCH RESULTS ARE HIGHER THAN PLATINUM RESULTS
	Gooch	Platinum	
Crude sewage.....	241	234	3
	636	632	1
	404	386	5
Settled sewage.....	186	164	12
	223	208	7
	103	78	24
Septic sewage.....	112	96	14
	146	122	16
	86	60	30
Coarse-grain filter.....	142	136	4
	211	196	7
	193	178	8
Settled effluents of coarse-grain filter.....	67	46	31
	89	70	21
	33	22	33
Average.....			13

#### THE INDIRECT DETERMINATION OF THE VOLATILE SUSPENDED MATTER.

It has been the practice for many years to ignite the dried and weighed residue upon evaporation of filtered and unfiltered samples, the loss in weight being taken to indicate the amount of organic matter present. The indirect determination of the volatile suspended matter is subject to a number of errors, as is well understood. In order that the residue shall be as free as possible from water of crystallization in the presence of incrustants, and to prevent to a considerable degree the loss of carbonic acid from magnesium salts also, and to convert all alkaline earth chlorides to the less volatile carbonates, it is customary to add a slight excess of sodium carbonate. Under such conditions, the residues do not usually contain water of crystallization, which will not be again taken up when the residues are moistened with water and evaporated. Owing to the complex and uncertain composition of the basic magnesium carbonate formed during the evaporation with sodium carbonate, considerable error is

<sup>1</sup>"Report of Committee on Standard Methods," *Jour. Infect. Dis.*, 1905, Suppl. No. 1, p. 44.

introduced upon ignition when magnesium is present, due to the loss of carbonic acid and some combined water.<sup>1</sup>

From the careful studies upon the "loss on ignition" made at Lawrence in 1890, it appears that the loss is approximately equal to 83 per cent of the weight of the magnesium (Mg) present. To determine further data upon this point, solutions of magnesium sulphate, in amount corresponding to 30, 60, 90, and 120 parts per million magnesium (Mg), were respectively treated with sodium carbonate in slight excess, evaporated in platinum, dried, and weighed. The dishes were then ignited for two minutes in the radiator, cooled, moistened with water, re-evaporated, dried, and weighed. Magnesium sulphate was used in this study for convenience. Since the magnesium of the alkalinity is also precipitated as a basic carbonate by heat and sodium carbonate, the end result of soda ash treatment is practically the same, whether or not, under natural conditions, incrustant magnesium is the only form in which the magnesium is present in the sewage.

From the results which appear in the table below, it is shown that the loss in weight, due to the loss of carbonic acid and combined water from the dried basic magnesium carbonate, is about 50 per cent of the total magnesium (Mg) present. As the average amount of magnesium (Mg) in the Columbus sewage is about 60 parts per million, the ignition of the residue of 50 c.c. of sewage entails a loss of 0.0015 gram, or 30 parts per million. These errors tend to balance each other in the unfiltered and filtered samples, so that the suspended loss is not affected to as marked a degree as might at first appear. The volatile matters, on the other hand, are obviously somewhat distorted.

TABLE 4.

LOSS IN WEIGHT UPON IGNITION OF MAGNESIUM SULPHATE AND SODIUM CARBONATE IN RADIATOR.  
(Average of Two Experiments.)

Mg. Parts Per Million	Actual Weight Mg (Gram)	Actual Weight of Magnesium Sulphate and Sodium Carbonate	Loss in Weight after Two Min. Igni. (Gram)	Per Cent Loss in Weight
30	0.015	0.0146	0.0009	60
60	0.030	0.0285	0.0013	43
90	0.045	0.0413	0.0021	47
120	0.060	0.0556	0.0027	45
Average	.....	.....	.....	49

#### THE DIRECT DETERMINATION OF VOLATILE SUSPENDED MATTER BY THE GOOCH CRUCIBLE METHOD.

The evidence seems to be very clear that the Gooch crucible method furnishes data upon volatile suspended matter which more

<sup>1</sup>Mass. State Board of Health Report, 1890, 2, p. 715.

correctly represent the actual conditions, since in this method the actual loss in weight resulting from the burning of the organic constituents is measured by a primary process.

When experiments were first begun looking to the feasibility of the adoption of the Gooch crucible, attention was soon directed to the great discrepancies existing between the volatile matter results obtained by the Gooch, as compared with those given by the platinum method. The extent of the variability of volatile suspended matter results by the two methods may be noted from the results in the following table:

TABLE 5.  
COMPARATIVE VOLATILE SUSPENDED MATTER RESULTS BY GOOCH AND PLATINUM METHODS.

SOURCE OF SAMPLE	VOLATILE SUSPENDED MATTER		
	Parts per Million		Per Cent Which Platinum Is of Gooch
	Gooch	Platinum	
Crude sewage.....	132	06	72
	205	28	63
	174	148	85
Settled sewage.....	107	70	65
	124	06	77
	65	32	40
Septic sewage.....	74	50	76
	70	40	51
	74	46	62
Effluent of coarse-grain filter..	45	28	62
	50	30	60
	48	24	50
Settled effluent of coarse-grain filter.....	23	2	17
	25	4	16
	23	4	17
Average.....	83	40	50

FACTORS ASSOCIATED WITH THE VARIABLE RESULTS OF THE INDIRECT  
EVAPORATION METHOD AS EXPLANATORY OF THE  
DISCREPANCIES EXISTING BETWEEN THE  
GOOCH AND PLATINUM METHODS.

The results given in the preceding table, showing such wide variation in the volatile suspended matter figures for the same example, but examined by two different methods, suggest that one of them must be open to serious criticism. Since in the Gooch method we are dealing with the actual suspended matter, it would appear that the discrepancies must be looked for in the indirect method, which, as is well understood, is subject to many inherent sources of error when it becomes a question of the absolute significance of the results.<sup>1</sup>

<sup>1</sup>Mass. State Board of Health Report, 1800, 2, p. 715.



One of the chief variables in the standard platinum method, which affects the indirect volatile suspended matter determination, refers to the incompleteness and uncertain character of the ignition. These irregularities have to do with the temperature of ignition, the time of ignition, and the relative amount and character of the mineral salts present. It is clear that the temperature of ignition must not be raised above the volatilization point of chlorides, nor above that at which oxygenated compounds become deoxygenated; and, as is well known, it is to avoid such complications that the radiator is used. Under such limitations it is apparent that the completeness of the combustion of the carbon will, in a large measure, depend upon its state of division, and perhaps upon the more important factor associated with the occluding property of the inorganic salts present. A close examination of residues ignited in platinum, according to the standard procedure, will show small particles of carbon remaining unoxidized. Since a complete ignition is understood to yield a residue white in color, save for the possible stain of oxide of iron, it is obvious that the present practice in regard to loss on ignition falls short of complete oxidation. Owing to the occlusion by the predominating mineral salts in residues from hard sewages, the loss on ignition results tend to be low, as already noted. To illustrate the

TABLE 6.

RELATIVE EFFECT OF SECOND IGNITION UPON THE TOTAL AND DISSOLVED VOLATILE MATTER.

CHARACTER OF SAMPLE	VOLATILE MATTER					
	Parts per Million				Per Cent Which First Ignition Is of Second Ignition	
	Total		Dissolved			
	1st Ignition	2d Ignition	1st Ignition	2d Ignition	Total	Dissolved
Crude sewage.....	72	92	44	54	78	81
	104	126	76	88	82	86
	98	106	78	82	91	95
Settled sewage.....	70	96	42	58	73	77
	178	200	114	122	89	93
	160	186	114	134	86	85
Septic sewage.....	74	110	40	58	67	70
	162	188	114	126	86	90
	182	208	126	156	88	81
Effluent of coarse-grain filter.....	68	98	40	52	70	77
	70	100	40	48	70	83
	66	108	40	50	61	80
Average.....					78	83

extent to which the occlusion of volatile matter by the mineral constituents of the residue is a factor in controlling the loss in weight on ignition, and also to illustrate what is thought to be one of the greatest sources of error in the determination of volatile suspended matter by the platinum method, the preceding table has been prepared, in which are presented data representative of a large number of determinations upon the point under discussion.

In the *Fourth Report of the Royal Bureau of Sewage Disposal and Water Purification* at Berlin,<sup>1</sup> in an account of the investigations at Cologne, Germany, the method used for the determination of the total and volatile suspended matter is given in detail. As refers to volatile matter, their method of ignition merits special comment, since it aims to ensure a very complete ignition, eliminating the occlusion factor to which we have already referred. In brief, the technique involves repeated ignitions, followed by applications of distilled water, until the small particles of carbon occluded by the mineral matter have entirely disappeared, leaving a pure white residue. Usually from four to six ignitions were necessary. To effect the ignition, the dish was moved cautiously back and forth over a free flame from a mushroom burner. As soon as considerable charring was noted, the residue was moistened with water, the water evaporated, and the ignition repeated as before. After the final ignition the residue was moistened with a few drops of ammonium carbonate solution, and then slightly warmed, in order to take up again the carbonic acid which might have been driven off in spite of the cautious heating.

Although, in the presence of magnesium, considerable error seems inevitable, yet this method appears to be an improvement over the ignition practices in this country, in which the occlusion of particles of organic matter by the mineral constituents of the residue is often a factor of considerable moment. To learn the extent to which the increased loss in weight could be attributed to the loss of carbonic acid from basic magnesium carbonate in the presence of sodium carbonate, a second ignition was made of the residues obtained in the magnesium experiments already described. It appears from these results that there may be an additional loss of about 8 per cent, or about five parts per million for 60 parts of magnesium (Mg), which is not to be traced

<sup>1</sup>Report of Royal Bureau of Sewage Disposal and Water Purification, Berlin, Germany, 1904.

to the more perfect combustion of the organic matter. Such errors as this obviously affect the total loss on ignition values rather than the volatile suspended matter results, as before mentioned. The effect of a second ignition upon basic magnesium carbonate is shown in the following table:

TABLE 7.  
EFFECT OF SECOND IGNITION UPON BASIC MAGNESIUM CARBONATE.

(Mg) PARTS PER MILLION	ACTUAL WEIGHT Mg (GRAM)	LOSS IN TOTAL WEIGHT (GRAM)		PER CENT TOTAL LOSS IN WEIGHT	
		First Ignition	Second Ignition	First Ignition	Second Ignition
30.....	0.0150	0.0009	0.0010	60	67
60.....	0.0300	0.0014	0.0018	47	60
90.....	0.0450	0.0019	0.0023	43	51
120.....	0.0600	0.0023	0.0027	38	43
Average.....				47	55

To determine the effect of further treatment with water and a second ignition in the radiator, residues from the usual processes have been so treated. Upon the reapplication of distilled water, and as a result of the second ignition, we have obtained residues containing no visible particles of carbon. How far the elimination of the occlusion factor will increase the value of the suspended loss on ignition results, the following comparative data may serve to illustrate. These results also indicate, in a general way, that a second application of water and a reignition of the residue from the first ignition would greatly enhance the value of loss on ignition data, bearing in mind the magnesium factor already discussed.

TABLE 8.  
RELATIVE RESULTS BY GOOCH AND PLATINUM METHODS SHOWING EFFECT OF SECOND IGNITION.

CHARACTER OF SAMPLE	VOLATILE SUSPENDED MATTER, PARTS PER MILLION		
	Platinum Method		Gooch Method
	Two Minute Ignition	Second Ignition Three Minutes	
Crude sewage.....	28	38	43
".....	82	92	101
Settled sewage.....	28	38	44
".....	34	52	53
Effluent coarse-grain fil- ter.....	30	52	50
Settled effluent of coarse- grain filter.....	28	46	44
	4	28	28
	4	30	30

## SUMMARY AND CONCLUSIONS.

The results of these studies seem to show, with considerable clearness, the practical applicability of the Gooch crucible to the determination of the amount and character of the suspended matter in sewages and in sewage effluents. The advantages of the Gooch crucible method consist in accuracy, speed, and completeness of filtration, and the comparatively small number of operations entailed in its technique. Compared with the usual platinum evaporation method, and aside from the admitted superiority of a direct process, the use of the Gooch crucible very materially reduces the time and labor involved in manipulation. It eliminates the errors of the evaporation method, relating to uncertain amounts of water of crystallization in the dried total and dissolved residues, respectively, and the liability to a variable loss during ignition, due to deoxygenating decomposition and volatilization of certain mineral salts. As indicated also, the Gooch method not only gives results the equal of those obtained by the platinum method, but, when desired, it affords a practical means for estimating the amount of very finely divided suspended matter usually contained in sewages.

In the case of the platinum evaporation method, our results suggest the strong advisability that a second ignition should be included in the standard procedure, both for sewage and for polluted water, since by this means a more nearly perfect combustion of the organic matter seems assured at radiator temperature. An initial partial ignition of the residue, followed by the application of a small amount of distilled water, evaporation, and a second ignition, it is thought, would ensure a very complete oxidation of the organic matters at a low radiator temperature, thus obtaining the most efficient result at a temperature far below the volatilization point of mineral matter. Further, it is considered that asbestos could be used to advantage as a filtering material in sewage work, in order to effect a more complete removal of finely divided suspended matter than is possible by the use of filter paper.

During the investigations at Columbus, over 500 determinations of suspended matter were made by the Gooch crucible method, in samples ranging from the crude sewage containing as high as 1,500 parts per million of total suspended matter, to settled effluents from



sprinkling filters containing as low as 20 parts per million of suspended matter, duplicate results showing that the method was at least applicable to this range of suspended matter in amount and character.

Such being the range of the applicability of the Gooch crucible method and in view of the several advantages it possesses over the indirect platinum method for the determination of suspended matters, the conclusion seems justified that, for practical routine work upon sewage, especially in investigations relating to physical questions involved in sewage purification, the Gooch crucible method deserves recognition as a standard method.

In conclusion the writers desire to express their thanks to Mr. Julian Griggs, chief engineer of the Board of Public Service, Columbus, Ohio, through whose courtesy they have been permitted to publish the results of these studies in advance of the regular testing-station report. Further, they desire heartily to acknowledge the valuable criticisms of Mr. George W. Fuller, consulting engineer, and the helpful suggestions of Mr. George A. Johnson, engineer in charge of the Columbus Testing Station.

## THE RESISTANCE TO DECOMPOSITION OF CERTAIN ORGANIC MATTERS IN SEWAGE.

H. W. CLARK.

THIS investigation, in regard to the resistance to decomposition of certain organic matters, was begun in order thoroughly to comprehend the conditions prevailing in certain sand filters which had received sewage for periods varying from 10 to 17 years. Studies of the work accomplished by these filters during these many years of operation show that only about 55 per cent, of the nitrogen in the sewage applied to them has appeared in their effluents. Studies of municipal areas, moreover, receiving a fresher sewage, have shown that only about 30 per cent of the applied nitrogen appears in the effluents from these areas. Much of the remaining nitrogen is set free by nitrogen-liberating bacteria; but a small percentage of the nitrogenous matters is resistant to bacterial actions and accumulates within the filter—from 4 to 8 per cent in the experimental filters at Lawrence.<sup>1</sup>

The filters under discussion had stored, in the period stated, enough organic matter seriously to impair their satisfactory operation; that is, the upper layers of these filters had become clogged to such an extent that it was necessary to break through these layers and to ridge and trench the surface of the filters in order that good purification of the applied sewage might be obtained. It was hoped that, by this surface treatment, the sand piled in ridges would lose the larger part of its stored organic matters by the work of the bacteria present, thus given a chance to work over the organic matter stored in the sand, rather than the organic matter in the sewage applied daily.

From the sand of the ridges, arranged in this way upon the three filters chiefly studied, differing amounts of nitrogen disappeared. From the ridges upon two filters constructed of coarse sand, 46 per cent of the stored nitrogen disappeared in three months, and from the ridges upon a filter constructed of comparatively fine sand, only

<sup>1</sup> For a fuller account of this work, see *Report of Massachusetts State Board of Health*, 1904.

about 10 per cent disappeared in an equal period. The residual nitrogenous matters remaining upon the sand in these ridges remained, however, after this first quick and easy removal, month after month without change, during weather favorable to bacterial activity in the filters.

Following this work, small filters of sand, taken from the upper portions of the three sand filters under investigation and rich in organic matter, were constructed. These small filters, started early in May, 1904, were placed in the laboratory and kept under conditions favorable to nitrification. Each of these filters was flooded with water, and the rate of operation of each was 30,000 gallons per acre daily. Nitrification started immediately in the filter constructed of coarse sand, and at the end of a week of operation, the effluent of this filter contained 15 per cent of nitrates per 100,000. In the other two filters, nitrification also started quickly, and the nitrates in the effluent of each became comparatively high very soon after the beginning of their operation. The nitrates remained high for about two months, and much of the nitrogenous organic matter on the sand was removed during this period; from the filter of coarse sand, 70 per cent, and from the remaining filters 55 and 30 per cent, respectively. At the end of this period of quick nitrification, however, although conditions were the same, nitrification practically ceased, and the organic matter upon the sand, both nitrogenous and carbonaceous, remained constant in amount. Determinations of the nitrogen in each filter and in the effluent of each were made, and it was shown that of the total amount of nitrogen removed from the sand of each filter during its period of operation a very varying amount had passed off in the three effluents; in the effluent of one, only 18 per cent of the total amount, in the effluent of the second, 23 per cent, and in the effluent of the third, 71 per cent. That is to say, much nitrogen lost from the sand passed away in the air, as the result of the work of the nitrogen-liberating bacteria undoubtedly present in each filter, the largest amount being liberated from the filter of coarsest sand.

After nitrification had been low in each filter for a period of several months, and the organic matter upon the sand had remained at a practically constant figure, as shown by various analyses, attempts

were made to increase nitrification, by dosing each filter with chemicals or cultures of bacteria, to induce the activity of the nitrifying organisms. Small amounts of sewage were added, also, in order to be sure that nitrifying bacteria were present in each filter, but this was without effect in increasing nitrification. Further, in order to prove that the filters were capable of producing nitrates, if easily nitrified matter was applied to them, a solution of ammonium chloride and sodium carbonate was applied to one filter, and peptone to a second filter. Following the application of these substances, nitrification again became high in each filter, but ceased quickly when these bodies were omitted from the water applied.

In order to study further the character of the matter stored in these filters, determinations of the total organic matter present were made. By this means, it was found that the nitrogen present was a very small and varying per cent of the total organic matter, probably not more than 3 per cent, judging from many analyses, and that the principal clogging matter upon the sand was carbonaceous and of the nature of cellulose. The investigation is still under way, but so far shows clearly that there is in ordinary domestic sewage a certain small percentage of exceedingly stable carbonaceous and nitrogenous matter, that, accumulating year after year, seriously impairs the work of these filters; that these matters are but slowly affected by the ordinary bacterial actions depended upon to oxidize, or cause the disappearance, of organic matter; that even when this clogged sand is taken from the filters and placed under conditions favorable to nitrification, as in the small filters described in this paper, a considerable portion of the organic matter remains unaffected; that the larger part of this matter is of a carbonaceous rather than of a nitrogenous nature. The practical bearing of this is that it indicates clearly that from the ordinary intermittent sand filter for the disposal of sewage, sand will, in most instances, eventually have to be removed, notwithstanding careful oversight and good operation of such filters.



## THE COLLECTION AND PRESERVATION OF SAMPLES OF SEWAGE FOR ANALYSIS.

STEPHEN DEM. GAGE AND GEORGE O. ADAMS.

It is well known that the composition of the sewage from any source is not uniform, varying with the time of day, the amount of surface water entering the sewers, and also with the temperature and with the time which elapses between collection and analysis. The variations in the composition due to these different factors have been frequently discussed, and it is unnecessary to enter into their further consideration at this time.

In the design and control of sewage disposal systems it is of the first importance to obtain the true average composition of each day's flow of sewage, by collecting series of samples covering 24 hour periods. The number of such samples which may be collected is usually limited, however, by the capacity of the testing laboratory to make the necessary analytical determinations before decomposition has started, and also unless exceptional facilities for the transportation of samples are provided, the time elapsing between collection and analysis is sufficient in many cases to affect seriously the accuracy of the results. If some simple and effective method of preserving these samples were used, the capacity of the laboratory to handle a series of samples would be greatly increased, as the work could be extended over several days; and there would also be an increase in the value of the analytical results, since the samples when analyzed would be in the same condition as when collected.

In the control of many municipal sewage areas it is customary to send samples to a central laboratory at stated times, and from the results of the analyses of these samples to interpret the purification accomplished by the area. That these samples are not representative is well known, but hitherto attention has been centered on improving the analytical methods, to the neglect of methods of sampling. Monthly or semi-monthly analyses of average samples, made up of small portions collected two or three times daily and preserved in some manner by the filter attendants, would yield more representa-

tive results without imposing any great burden on the filter attendants.

Two methods of preserving samples are open to us: the use of cold and the use of chemicals. The use of cold, while fairly satisfactory for short periods, is limited by the fact that ice is not readily obtainable at sewage disposal works, is difficult to handle, increases transportation charges, and even when obtainable is less convenient than chemical treatment. It is generally understood that the decomposition which takes place in sewage and in the effluents from sewage filters is largely, if not entirely, due to bacteriolytic action. To be thoroughly effective, then, the chemical employed as a preservative should immediately check all bacterial action, should be comparatively cheap and easily obtainable, and, above all, should not interfere with any of the chemical determinations.

The report of the Committee on Standard Methods of Water Analysis,<sup>1</sup> discussing the time which may be allowed to elapse between collection and analysis of samples, states (p. 14) that six hours is the maximum which may be permitted for sewages and the effluents from sewage purification systems. On p. 15, the report says: "If sterilized by the addition of chloroform, formaldehyde, mercuric chloride, or some other disinfectant, samples for chemical and microscopical examination may be allowed to stand for longer periods. . . ."

Neither formaldehyde or mercuric chloride conforms to the requirements of an ideal sewage preservative, in that both interfere with certain of the chemical determinations, formaldehyde giving a yellow color with Nessler reagent in the free ammonia determinations and reducing the permanganate of potash in the oxygen consumed process, and mercuric chloride interfering with the determinations of both chlorine and solids. For many years, mercuric acetate has been used at the Lawrence Experiment Station for preserving average samples of sewage; but while the disinfecting action of this has been entirely satisfactory, it is not readily obtained, and interferes with the determination of the solids.

As a result of studies made at the Lawrence Experiment Station during the past year, it has been found that chloroform and carbon-bisulphide most nearly fulfil the ideal conditions. Both are readily

<sup>1</sup> *Jour. Infect. Dis.*, 1905, Supplm. No. 1 p. 1.

obtainable, are sparingly soluble in water, and, being heavier than water, do not evaporate rapidly, requiring only a small amount in each sample. Chloroform, however, is the more satisfactory of the two, being more pleasant to use, thoroughly effective, and, so far as

TABLE 1.

THE RESULTS OF CHEMICAL DETERMINATIONS ON DUPLICATE SAMPLES WITH AND WITHOUT THE PRESENCE OF CHLOROFORM.

	FREE AMMONIA		ALBUMINOID AMMONIA	
	With Chloroform	Without Chloroform	With Chloroform	Without Chloroform
Effluent sand filter.....	0.0434	0.0426	0.0220	0.0220
Effluent trickling filter.....	0.0750	0.0500	0.1460	0.1340
Effluent contact filter.....	0.1120	0.1040	0.0960	0.1020
Effluent septic tank.....	1.0000	1.0800	0.1480	0.1360
Raw sewage.....	0.6600	0.6800	0.1500	0.1260
	2.2000	2.2000	0.1900	0.2300
	5.3000	5.1000	0.4400	0.4300

## NITROGEN AS

	NITRATES		NITRITES	
	With Chloroform	Without Chloroform	With Chloroform	Without Chloroform
Effluent sand filter.....	2.94	2.94	0.0032	0.0032
Effluent trickling filter.....	2.94	3.11	0.0028	0.0032
Effluent contact filter.....	1.51	1.60	0.0040	0.0040
Effluent septic tank.....	1.43	1.60	0.0020	0.0020
Raw sewage.....	0.02	0.02	0.0000	0.0000
	1.03	1.07	.....	.....

	CHLORINE		OXYGEN CONSUMED	
	With Chloroform	Without Chloroform	With Chloroform	Without Chloroform
Effluent sand filter.....	10.20	10.20	0.37	0.37
Effluent trickling filter.....	8.42	8.42	1.51	1.60
Effluent contact filter.....	7.50	7.50	1.58	1.18
Effluent septic tank.....	10.58	10.58	1.20	1.26
Raw sewage.....	0.60	0.60	0.82	0.74
	8.80	8.80	3.64	3.60
	0.20	0.20	1.12	1.30
	10.10	10.10	5.45	5.70

## ORGANIC NITROGEN (KJELDAHL).

	With Chloroform	Without Chloroform
Effluent sand filter.....	0.2698	0.2936
Effluent trickling filter.....	0.2936	0.2558
Effluent contact filter.....	0.1788	0.1779
Effluent septic tank.....	0.1878	0.1779
Raw sewage.....	0.2731	0.2772
	0.2706	0.2501
	0.2854	0.2837
	0.4400	0.4300

we have been able to determine, not interfering with any of the chemical determinations. Carbon-bisulphide interferes with the determination of nitrates by the aluminum method, and is decomposed by some sewages into hydrogen sulphide or free sulphur, which interferes with some of the chemical determinations, and renders it less effective in preventing bacterial decomposition. In order to prove that chloroform had no effect on the results of the various analytical determinations, many determinations were made of samples with and without the addition of that substance. Some of these comparative determinations are shown in Table 1, from which it is seen that, while the results vary slightly among themselves, they are well within the limits of error of sampling and analysis.

Experiments were then made to determine the proportion of chloroform necessary to destroy the bacteria, or at least to prevent them from multiplying. Various amounts of chloroform were added to bottles of sewage from which the suspended matter had been removed by filtering through paper, and the samples were allowed to stand in the laboratory, determinations of the numbers of bacteria being made at frequent intervals. In these experiments it was found that between 5 and 10 c.c. of chloroform in one gallon of sewage would control the bacteria, this amount making practically a saturated solution. In practice based on further experiments, it has been found advisable to add somewhat more chloroform, and to recover the excess when the analyses are complete. The recovery is easily

TABLE 2.

THE EFFECT OF DIFFERENT AMOUNTS OF CHLOROFORM ON THE BACTERIA IN ONE GALLON OF SEWAGE.  
(Bacteria per c.c.)

Elapsed Time	No Chloroform	2 c.c. of Chloroform	5 c.c. of Chloroform	10 c.c. of Chloroform
Start.....	1,270,000	1,300,000	370,000	10,000
1 day.....	1,650,000	760,000	3,000	300
2 days.....	2,200,000	1,160,000	6,500	16
3 ".....	650,000	1,140,000	75	16
4 ".....	900,000	1,690,000	2,000	90
5 ".....	730,000	2,000,000	3,900	75
7 ".....	830,000	3,050,000	1,600	60
9 ".....	820,000	7,200,000	22,400	180
11 ".....	480,000	5,250,000	140	30
15 ".....	190,000	3,120,000	5,800	5
18 ".....	80,600	6,840,000	6,800	30
21 ".....	500,000	10,800,000	108,000	90
28 ".....	280,000	15,120,000	4,600	100
35 ".....	380,000	21,650,000	90	42
42 ".....	14,000	6,770,000	28,600	32
49 ".....	71,400	14,040,000	10,200	75
63 ".....	24,500	2,250,000	280	60



made by inverting the samples over a funnel in another bottle filled with water, when the chloroform sinks into the lower bottle, and may be distilled when a sufficient amount has accumulated. The effectiveness of different proportions of chloroform in controlling bacterial life is shown in Table 2.

As a method of preservation would, in practical use, be subjected to considerable variation in temperature, experiments were made to determine the effectiveness of chloroform in preserving samples which were allowed to stand at different temperatures. Samples of sewage, from which the suspended matter had been removed by filtering through paper, were treated with chloroform and incubated at 10°, 20°, and 30° C., respectively, analyses being made at frequent intervals. The results of the various determinations fluctuated more or less, but no regular change appears to have taken place in any of the samples, and it is probable that the fluctuations were due to errors in obtaining the proper sample from the bottle and in the chemical methods. The most noticeable change was in the oxygen consumed, and part of this may have been due to oxidation during the numerous shakings incident to removing samples for 13 analyses. The various analytical results in one experiment with sewage treated with chloroform standing at different temperatures are shown in Tables 3, 4, and 5.

In order to test the applicability of the method to sewages which had been more or less completely purified, experiments were made in

TABLE 3.  
SEWAGE TREATED WITH CHLOROFORM STANDING AT 10° C.

Elapsed Time	Free Ammonia	Albuminoid Ammonia	Organic Nitrogen (Kjeldahl)	Ratio of Albuminoid Nitrogen to Kjeldahl Nitrogen	Oxygen Cons.	Bacteria per c.c.
Start.....	5.10	0.44	0.82	43.5	5.45	39,000
1 day.....	5.30	0.43	0.81	43.6	5.70	0
2 days.....	4.50	0.43	0.78	45.3	4.60	24
3 ".....	5.60	0.46	0.74	51.1	5.45	16
4 ".....	5.60	0.45	0.92	40.1	4.60	3
5 ".....	5.00	0.44	0.82	44.0	5.30	70
7 ".....	4.90	0.43	0.78	45.3	4.70	22
10 ".....	5.20	0.39	0.91	35.2	4.85	41
15 ".....	4.70	0.42	0.74	46.5	4.75	10
17 ".....	5.20	0.47	0.84	45.8	4.20	14
22 ".....	4.80	0.38	0.83	39.5	4.65	16
28 ".....	4.80	0.42	0.83	35.5	4.85	27
36 ".....	5.00	0.39	0.67	47.8	4.30	25
42 ".....	5.70	0.41	0.74	45.4	4.25	25

TABLE 4.  
SEWAGE TREATED WITH CHLOROFORM STANDING AT 20° C.

Elapsed Time	Free Ammonia	Albuminoid Ammonia	Organic Nitrogen (Kjeldahl)	Ratio of Albuminoid Nitrogen to Kjeldahl Nitrogen	Oxygen Cons.	Bacteria per c.c.
Start.....	5.10	0.44	0.82	43.5	5.45	39,000
1 day.....	4.70	0.42	0.81	42.5	5.20	200
2 days.....	4.60	0.41	0.73	46.0	4.45	26
3 ".....	5.00	0.45	0.74	50.0	5.35	17
4 ".....	5.30	0.50	0.94	43.6	4.45	11
5 ".....	5.50	0.43	0.81	43.6	3.55	20
7 ".....	5.20	0.45	0.81	45.6	4.60	25
10 ".....	5.20	0.43	.....	.....	4.50	28
15 ".....	5.30	0.41	0.81	41.5	4.60	15
17 ".....	5.20	0.42	0.85	41.0	4.00	6
22 ".....	4.80	0.40	0.75	45.9	4.30	20
28 ".....	4.70	0.44	0.78	46.3	4.55	21
36 ".....	5.30	0.42	0.68	50.6	4.20	55
42 ".....	5.20	0.41	0.84	40.0	4.40	28

TABLE 5.  
SEWAGE TREATED WITH CHLOROFORM STANDING AT 30° C.

Elapsed Time	Free Ammonia	Albuminoid Ammonia	Organic Nitrogen (Kjeldahl)	Ratio of Albuminoid Nitrogen to Kjeldahl Nitrogen	Oxygen Cons.	Bacteria per c.c.
Start.....	5.10	0.44	0.82	43.5	5.45	39,000
1 day.....	5.50	0.45	0.82	45.0	4.45	300
2 days.....	5.10	0.39	0.79	40.7	3.95	26
3 ".....	4.70	0.42	0.72	47.9	4.30	24
4 ".....	5.40	0.48	0.84	46.9	4.30	23
5 ".....	6.20	0.43	0.82	43.1	2.50	30
7 ".....	5.20	0.40	0.75	43.7	4.30	23
10 ".....	5.20	0.40	0.88	37.3	4.40	40
15 ".....	4.90	0.41	0.77	43.6	4.35	2
17 ".....	4.40	0.48	0.83	47.5	3.75	8
22 ".....	5.30	0.42	0.89	38.7	3.95	16
28 ".....	5.60	0.41	0.74	45.4	4.30	17
36 ".....	5.20	0.44	0.84	43.0	4.00	26
42 ".....	5.20	0.41	0.78	43.1	4.15	31

which effluents from a sand filter, a trickling filter, a contact filter, and septic tank were treated with an excess of chloroform, to determine whether the destruction of the bacteria by the antiseptic would prevent chemical decomposition, as had been noted in the raw sewages. The same fluctuations occurred in these samples as were previously noted with sewage samples. The variations, however, were greater than in the sewages above, because while the former contained little suspended matter, there was a considerable amount in the latter, making it more difficult to obtain a true sample. The results are shown in the following table:

TABLE 6.

TABLE SHOWING PRESERVATIVE ACTION OF CHLOROFORM ON SEPTIC SEWAGE, AND THE EFFLUENTS FROM SAND, CONTACT, AND TRICKLING FILTERS.

ELAPSED TIME	AMMONIA		ORGANIC NITROGEN (KJEL- DAHL)	RATIO OF ALBUMINOID NITROGEN TO KJELDAHL NITROGEN	OXYGEN CONS.	NITROGEN AS		BACTERIA PER C.C.
	Free	Alb.				Nitrates	Nitrites	
EFFLUENT SAND FILTER.								
Start.....	0.9500	0.1460	0.2936	40.8	0.42	1.48	0.2000	72,000
4 days.....	0.9750	0.1340	0.2558	42.9	0.96	1.81	0.0000	108,000
8 ".....	0.8750	0.1400	0.2378	48.3	1.01	1.77	0.0000	200
15 ".....	0.8400	0.1460	0.2608	45.9	1.05	0.91	0.0600	600
22 ".....	0.7600	0.1740	0.2698	52.3	1.00	1.18	0.0000	98
32 ".....	.....	.....	.....	....	....	1.68	0.0000	....
EFFLUENT SAND FILTER.								
Start.....	0.0426	0.0220	.....	....	0.37	2.94	0.0032	1,900
3 days.....	.....	.....	.....	....	0.39	3.11	0.0028	14
10 ".....	.....	.....	.....	....	0.30	2.69	0.0030	....
17 ".....	0.0378	0.0208	.....	....	0.39	3.11	0.0028	450
EFFLUENT TRICKLING FILTER.								
Start.....	0.1760	0.1880	0.3378	45.6	1.33	0.91	0.0130	80,000
4 days.....	0.0880	0.2040	0.4478	37.4	1.14	1.09	0.0050	76,000
8 ".....	0.0880	0.2160	0.3649	48.4	1.28	0.92	0.0006	1,000
15 ".....	0.1120	0.2160	0.4264	41.6	1.61	0.88	0.0050	1,170
22 ".....	0.1120	0.2200	0.3813	47.3	1.36	1.01	0.0020	720
32 ".....	.....	.....	.....	....	....	1.09	0.0014	....
EFFLUENT TRICKLING FILTER.								
Start.....	0.1040	0.1020	0.1779	47.0	1.18	1.60	0.0040	17,500
3 days.....	0.1120	0.0960	0.1878	49.8	1.58	1.43	0.0020	30,000
10 ".....	0.1320	0.1220	0.1911	52.4	1.36	1.55	0.0032	640
17 ".....	0.1340	0.1320	0.2075	52.1	1.32	1.48	0.0032	800
EFFLUENT CONTACT FILTER.								
Start.....	0.6800	0.1260	0.2501	41.3	0.74	1.07	0.1900	650,000
4 days.....	0.6600	0.1500	0.2706	45.5	0.82	1.03	0.1500	165,000
8 ".....	0.5400	0.1720	0.2919	48.3	0.85	0.89	0.0400	26
15 ".....	0.6400	0.1640	0.2583	52.0	0.87	0.72	0.0800	22
22 ".....	0.6400	0.1720	0.3050	46.2	0.84	0.84	0.0400	14
32 ".....	.....	.....	.....	....	....	0.95	0.0400	....
EFFLUENT SEPTIC TANK.								
Start.....	2.20	0.23	0.42	45.0	1.30	....	.....	80,000
4 days.....	2.20	0.19	0.34	45.9	1.12	....	.....	120,000
8 ".....	2.10	0.17	0.31	44.3	1.30	....	.....	85
15 ".....	1.90	0.20	0.30	55.2	1.44	....	.....	119
22 ".....	2.00	0.17	0.30	47.0	1.14	....	.....	143

In the foregoing the most marked percentage change noted was in the nitrites. In order to study these nitrite changes, further experiments were made in which mixtures of sewage and water containing

sodium nitrite were treated with chloroform. In these experiments the nitrites in the samples treated with chloroform were unchanged, which would seem to show that the changes occurring in the nitrites in the effluents noted above were due to chemical, rather than bacterial, action, a view recently advanced by Phelps<sup>1</sup> as the result of his studies of the action of contact filters. The results of one of these experiments are shown in the following table:

TABLE 7.  
NITRITES.

CONTROL			CHLOROFORM	
Elapsed Time	Parts per 100,000	Bacteria per c.c.	Parts per 100,000	Bacteria per c.c.
Start.....	0.0320	30,000	0.0320	30,000
2 days.....	0.0320	86,400	0.0320	55
5 ".....	0.0040	4,430,000	0.0320	18
9 ".....	0.0000	5,260,000	0.0320	27
12 ".....	0.0000	2,710,000	0.0320	50
16 ".....	0.0000	5,250,000	0.0320	19

It has been frequently stated in the preceding that the fluctuations in the analytical results were within the errors of sampling and analysis. In order to ascertain what these errors would be in actual practice, 10 complete analyses were made of a sample of Lawrence sewage, and 10 determinations of nitrates and nitrites were made on the effluent from a trickling filter which contained a considerable amount of suspended matter. In making these analyses the routine procedure was followed, the work being divided between two analysts. The possible errors are from two sources; those due to sampling, i. e., the error in measuring out the volume for each determination and in obtaining an aliquot portion of the suspended matter in the sample, and those existing in the analytical methods. The sampling errors are variable for the different determinations, since it is necessary to take different volumes of the sewage. In the determination of free and unfiltered albuminoid ammonia in this experiment 5 c.c. of the sewage were used, and, owing to the large amount of suspended matter, it was necessary to measure that volume in cylinders, which are less accurate than flasks or pipettes. In determining the filtered

<sup>1</sup> "Contributions from the Sanitary Research Laboratory and Sewage Experiment Station of the Massachusetts Institute of Technology," *Jour. Infect. Dis.* 1905, Suppl. No. 1, p. 61.



albuminoid ammonia 10 c.c. of the sewage were used, the measurements being made also in cylinders.

The volumes of sewage for the determination of organic nitrogen by the Kjeldahl method were measured in flasks, 20 c.c. of the unfiltered and 25 c.c. of the filtered sewage being taken. Samples of the sewage for oxygen consumed were measured in 25 c.c. flasks, and for chlorine in 50 c.c. flasks. In the determination of nitrates 100 c.c. were measured in a flask, boiled down to 25 c.c., made up to 50 c.c. in a Nessler tube, and 2 c.c. of this, measured in a pipette, diluted to 50 c.c. in a Nessler tube for reading. The nitrite determinations were made on 10 c.c., measured in a pipette and diluted to 50 c.c. in a Nessler tube.

The error in the free ammonia as determined by the average deviation from the mean of the 10 determinations was 5 per cent. The mean error in the albuminoid ammonia determinations was 8 per cent and 8.5 per cent, respectively, for unfiltered and filtered samples. For organic nitrogen determined by the Kjeldahl process, the average deviation from the mean was 2.5 per cent for unfiltered samples and 3.3 per cent for filtered samples. The error of the chlorine determinations was practically negligible, while that of the oxygen consumed process was 2.1 per cent. There was no error in the nitrite determinations, all of the 10 results being identical. The

TABLE 8.

THE VARIATION IN RESULTS OF 10 SEPARATE CHEMICAL ANALYSES.

	FREE AMMONIA	SEWAGE						TRICKLING FILTER EFFLUENT	
		ALBUMINOID AMMONIA		ORGANIC NITROGEN (KJELDAHL)		CHLORINE	OXYGEN CONSUMED	NITROGEN AS,	
		Unfiltered	Filtered	Unfiltered	Filtered			Nitrates	Nitrites
1.....	6.20	0.86	0.46	1.05	0.80	11.80	4.88	1.00	0.0080
2.....	6.70	0.08	0.53	1.08	0.95	11.90	4.02	1.15	0.0080
3.....	7.00	0.08	0.43	2.02	0.85	11.80	4.76	1.10	0.0080
4.....	6.80	1.04	0.43	2.01	0.87	11.91	4.68	1.09	0.0080
5.....	7.10	1.12	0.39	1.03	0.93	11.80	4.60	1.09	0.0080
6.....	7.20	1.14	0.41	1.04	0.93	11.85	4.72	1.11	0.0080
7.....	6.80	1.12	0.51	2.07	0.90	11.80	4.56	1.07	0.0080
8.....	5.70	1.06	0.53	1.02	0.92	11.80	4.64	1.09	0.0080
9.....	7.00	0.92	0.47	1.82	0.92	11.80	4.68	1.09	0.0080
10.....	6.30	0.92	0.50	2.02	0.92	11.80	4.52	1.07	0.0080
Average.....	6.68	1.01	0.47	1.97	0.92	11.84	4.70	1.10	0.0080
Maximum.....	7.20	1.14	0.53	2.07	0.90	11.91	4.92	1.10	0.0080
Minimum.....	5.70	0.86	0.39	1.82	0.85	11.80	4.52	1.00	0.0080

average deviation of the nitrate results from the mean of the 10 readings was 3.6 per cent. The results of the 10 complete analyses of the sewage and 10 determinations for nitrates and nitrites on the trickling filter effluent are shown in Table 8.

#### CONCLUSIONS.

It is well known that considerable changes may take place in the composition of sewage and the effluents from sewage filters during short periods, and that the elapsing of a few hours between the time of collection and analysis may seriously affect the results of the analyses. Furthermore, samples collected at occasional intervals from sewers or from the effluents of sewage disposal works do not represent the actual average composition of waters from these sources. To be of the greatest value, samples should be collected at frequent intervals, preserved in some manner to prevent chemical changes, and then mixed in aliquot portions to form an average sample for analysis. Experiments at the Lawrence Station have shown that chloroform is a good preservative to use under these circumstances. From 10 to 25 c.c. of chloroform may be added to a gallon bottle, and small samples of from 100 to 200 c.c., collected daily, may be placed in the bottle, which is tightly stoppered; the average sample so obtained may be analyzed after any stated period without fear of material change in the individual samples of which it is composed. The principle errors which may result from such treatment are a small change in the nitrites, and the difficulty arising from the settling out of suspended matter. The nitrite difficulty may be obviated by taking occasional samples and doing nitrite determinations only. Any objection as to precipitation while the average sample is being collected affecting the determination of soluble and suspended organic matter may be overcome by filtering and preserving a separate portion of the daily sample. If such methods be carried out in practice, it is believed that they will result in a more accurate knowledge of the composition of applied sewages and the effluents from sewage disposal works, and will yield more accurate information as to the purification accomplished by the different methods of sewage disposal.

## A READY METHOD OF PREPARING A SILICA TURBIDITY STANDARD.

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ALTHOUGH the candle or electric light turbidimeter has largely replaced comparison with silica standards as a laboratory method of determining the turbidity of water, these instruments, equipped with the usual short tube, are of use only where the turbidity is above 100, while the platinum wire method is applicable only in field work. Even there its use is limited to waters of neither very high nor very low turbidity, besides which there is not infrequently great difficulty in securing the necessary conditions of light. Direct comparison with silica standards is, therefore, the method which must be resorted to in most cases where the turbidity is below 100.

Of all standards used by the water analyst, the silica turbidity standard is the most difficult to prepare. Even after the very tedious preparation of the diatomaceous silica itself, the powder so obtained is not always of the required degree of fineness, thus necessitating standardization by the platinum wire method or by use of the candle turbidimeter, instead of simply adding a gram per liter as originally recommended, to secure a standard of 1,000 parts turbidity per million.

The above considerations led the writer, about a year ago, to examine a number of commercial products with the view of obtaining a satisfactory substitute for the diatomaceous silica, so difficult of preparation. Various polishing powders, advertised by the manufacturers as being made from diatomaceous earth, were first tried, but none of these possessed the requisite degree of fineness or uniformity. Finally a test was made of a toilet preparation sold under the name of "Pears' Precipitated Fuller's Earth," and this has appeared to meet all the practical requirements.

This powder is almost pure white in appearance, and standards prepared from it are indistinguishable from those made from diatomaceous silica secured by the method recommended originally by

Whipple and Jackson and incorporated in the "Report of the Committee on Standard Methods of Water Analysis," of this Association.<sup>1</sup> It has even seemed to the writer that the tendency to striation, noticeable chiefly in the higher standards, is less marked.

A rough analysis of the "precipitated fuller's earth" showed it to be largely a silicate of aluminum, rather than a true silica, and the term "precipitated" is probably not used in its chemical sense. The theoretical objection of its not being a strict silica does not seem to the writer to be a serious objection, since the powder shows no tendency to lump on standing, and standards made five months ago still maintain their original turbidity unaltered.

A request to the manufacturers, asking certain particulars concerning this preparation, especially as to whether the method of manufacture was such that uniformity of the product could be assured, brought no response, but several lots bought at different times were all perfectly uniform. As each package contains about 200 grams, gross, it is a simple matter to standardize each lot when purchased, and thereafter standards can be made quickly at any time. The writer has found, however, that a suspension of one gram per liter gives a standard of 1,000 in all of the samples tested; and it would therefore seem probable that this would regularly be the case, though, naturally, it would be advisable not to assume this to be so without actual testing of each new lot.

<sup>1</sup>*Jour. Infect. Dis.*, 1905, Supplm. No. 1, p. 1.



# THE SOLUBILITY OF CALCIUM CARBONATE AND OF MAGNESIUM HYDROXIDE AND THE PRECIPITA- TION OF THESE SALTS WITH LIME WATER.

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THE problem of water softening is attaining such great importance that it seems advisable at this time to re-examine carefully the principles and chemical data involved, in order to render them as correct and trustworthy as possible. Prominent among the questions to be settled are the solubilities of normal calcium carbonate and of magnesium hydroxide.

## SOLUBILITY OF NORMAL CALCIUM CARBONATE.

The values given for the solubility of calcium carbonate by the different authorities quoted in Comey's *Dictionary of Chemical Solubilities*<sup>1</sup> vary from 10 to 113 parts per million. About one-half of these authorities give values of more than 50 parts per million, but the high figures represent chiefly the results of early experiments, and are evidently erroneous. The figures which appear to be most reasonable may be divided into two groups, one placing the solubility between 20 and 36 parts per million, the other, between 10 and 16 parts per million.

TABLE I.  
GROUP I.

Authority	Date	Temperature C.	Solubility in Parts per Million
Chevalet <sup>2</sup> .....	1860	..	34
Hoffman <sup>3</sup> .....	1865	..	34
Peligot <sup>4</sup> .....	....	..	20
Weltzien <sup>5</sup> .....	1865	..	36

GROUP II.

Bineau <sup>6</sup> .....	1857	..	16.0
Schlosing <sup>7</sup> .....	1872	16.0	13.1
Holleman <sup>8</sup> .....	1893	8.7	10.0
Holleman <sup>8</sup> .....	1893	23.8	12.5
Kohlrausch and Rose <sup>9</sup> .....	1893	18.0	13.0

Various methods were employed by the different investigators to secure these results, as indicated in the subjoined notes. Those who

obtained values greater than 30 parts per million did not mention having taken any precaution against two of the greatest sources of error involved, namely, the influence of the  $\text{CO}_2$  of the air, and the action of the solution of  $\text{CaCO}_3$  upon the glass vessels. It seems significant that the figures of Bineau, who apparently first made allowance for these sources of error, and those of the modern experimenters, who followed his example, are all well under 20. The value most commonly accepted for the solubility of the normal calcium carbonate is 30 parts per million, although Kimberly, in a recent paper,<sup>10</sup> used 20 parts per million as the best value.

Several preliminary experiments to determine the solubility of normal calcium carbonate were conducted by one of us, in 1903. The pure salt was boiled with distilled water, free from  $\text{CO}_2$ , in flasks of Bohemian glass, and after standing for some days, protected from the  $\text{CO}_2$  of the air, the resulting solution was filtered and titrated with N/50 acid. The results so obtained were 30.5 parts per million by one set of experiments, and 25 parts per million by another. During the following year these experiments were repeated, giving in one case 23 parts per million and in another 13.5 parts per million. The last value was obtained when a flask of Jena glass was substituted for the Bohemian glass flasks.

In order to determine the reason for these discrepancies and to obtain correct and concordant results, the following experiments were performed:

Two liter flasks of Jena glass were each fitted with a one-hole rubber stopper, through which projected a short piece of glass tubing, drawn to capillary dimensions at its upper end. Into each flask was put about 900 c.c. of distilled water, absolutely neutral in reaction to methyl orange, and lacmoid. The contents of the flasks were boiled for two hours, in order to drive off the last trace of  $\text{CO}_2$  gas, and at the end of this time the stoppers were removed, and replaced, as quickly as possible, before any air could enter, by one-hole stoppers each bearing a U tube filled with beads, well moistened with fresh concentrated  $\text{NaOH}$  solution, the bend of the tube forming an effective seal. Flask 1 was cooled to  $37^\circ \text{C.}$  by allowing it to stand over night in an incubator adjusted to maintain that temperature, while Flask 2 was cooled to room temperature ( $21^\circ \text{C.}$ ). There was then quickly introduced into each flask 5 g.  $\text{CaCO}_3$ , prepared by thoroughly washing the best procurable chemically pure product with hot distilled water, and drying to constant weight at  $100^\circ\text{--}110^\circ \text{C.}$  After stoppering with solid rubber stoppers, the flasks were kept, respectively, at the temperatures mentioned, and were frequently well shaken throughout the experiment, in order to render solution complete. At intervals of 24 hours portions were withdrawn, quickly

filtered through paper, and 100 c.c. of the filtrate titrated with N/50  $\text{H}_2\text{SO}_4$ , using phenolphthalein and methyl orange as indicators. The results are shown in the following table:

TABLE 2.

FLASK 1.

TIME	TEMPERATURE ° C.	ALKALINITY	
		Phenol- phthalein	With Methyl Orange
24 hours.....	37.5	...	15.0
53 ".....	"	7.0	15.0
77 ".....	"	6.5	14.5

FLASK 2.

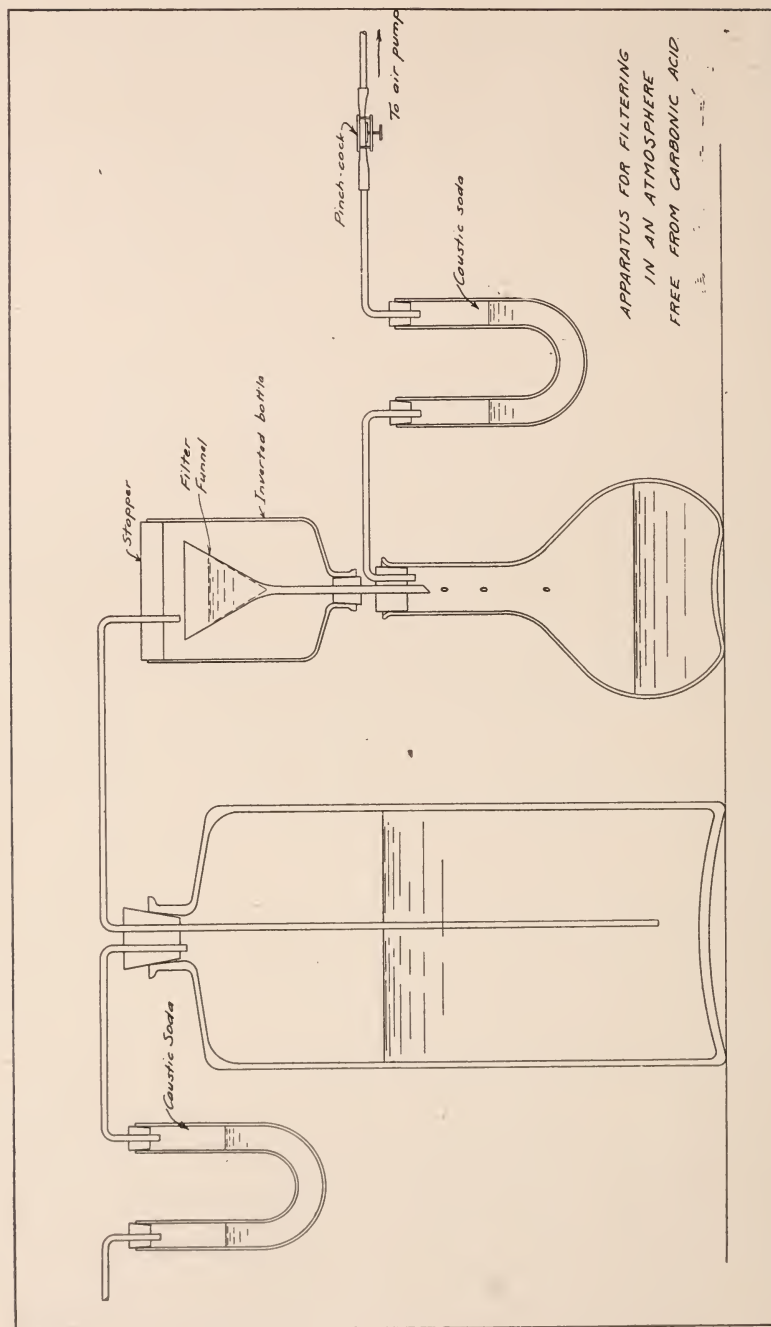
28 hours.....	23	6.5	14.0
53 ".....	22	6.0	13.0
76 ".....	20	6.5	13.0

The N/50  $\text{H}_2\text{SO}_4$  used in all this work was prepared from normal sulphuric acid, which was checked by titration against pure sodium carbonate, and by the gravimetric determination as barium sulphate. The dilution was done by means of a pipette and flask, which were calibrated against each other.

Another experiment, carried out in practically the same manner as those above described, at 20° C., gave 13 parts per million.

A value of 13 was also obtained by an experiment in which pure water, free from  $\text{CO}_2$ , was boiled, with an excess of chemically pure  $\text{CaCO}_3$ , for three hours in a flask of Jena glass, and allowed to stand with frequent agitation, protected from the  $\text{CO}_2$  of the air as above described, for a period of four days.

The alkalinity of the solution of  $\text{CaCO}_3$ , using phenolphthalein as indicator, should be equal to one-half of the value obtained by using methyl orange as indicator. It was found, however, when the solution was filtered in the air, that enough  $\text{CO}_2$  was dissolved to reduce the phenolphthalein figure. In order to overcome this difficulty, an apparatus was made to filter solutions out of contact with  $\text{CO}_2$ . (See diagram.) This apparatus, although accomplishing its object, consumed considerable time, as it was necessary, before attempting filtration, to remove from the apparatus every trace of  $\text{CO}_2$  gas by aspiration. The method finally adopted for securing the  $\text{CaCO}_3$  solution free from suspended particles, as well as  $\text{CO}_2$ , consisted in allowing all the  $\text{CaCO}_3$  to settle, after which 100 c.c. of the clear





supernatant solution could be drawn off with a pipette attached to a filter pump by a rubber tube. In every case in which this was done, all error due to the presence of  $\text{CO}_2$  in the air was avoided, and the phenolphthalein value was equal to just one-half the methyl orange value, as it should be. (See Table 2.)

In order to throw some light upon the high figures obtained in the preliminary work mentioned, experiments were conducted with flasks of Jena and Bohemian glass, in exactly the same manner as those last described, but omitting the addition of  $\text{CaCO}_3$ . The results of these blank tests are given in the following table:

TABLE 3.

TIME OF BOILING	TIME OF STANDING	ALKALINITY (PARTS PER MILLION)							
		Flask 1		Flask 2		Flask 3		Flask 4	
		P.*	M.†	P.	M.	P.	M.	P.	M.
1 hour.....	0 days	..	1.0	..	..	0.5	2.0	..	..
2 hours.....	4 "	..	..	..	..	..	..	10.5	12.5
2 ".....	3 "	..	..	0.75	1.5	..	..	..	..
3 ".....	0 "	0	..	..	..	4.0	6.5	..	..
4 $\frac{1}{2}$ ".....	0 "	0	1.5	..	..	5.5	9.5	..	..
4 $\frac{3}{4}$ ".....	5 "	0	2.5	..	..	7.0	10.0	..	..

\*Phenolphthalein.

†Methyl orange.

1 and 2 Jena glass flasks. 3 and 4 Bohemian glass flasks.

As the presence of small amounts of alkali in solution accelerates the action of water upon glass, and as the flasks used in the preliminary experiments were, with one exception, made of Bohemian glass, it can be readily seen why the figures first obtained were so high and variable. If, however, we subtract the average figure obtained in the blank experiments on Bohemian flasks, namely, 11.25, from the values obtained in the preliminary experiments, in which that kind of glass was used, we obtain values of 12 to 19 parts per million.

A better figure, however, is arrived at by subtracting from the results obtained in Jena flasks the corresponding blank, namely, 1.5, which gives us, in round numbers, 12 parts per million at  $22^\circ \text{C}$ ., and 14 parts per million at  $37.5^\circ \text{C}$ .

The figures for the solubility of normal calcium carbonate, i. e., from 12 to 14 parts per million, were corroborated by other experiments described later, in which calcium bicarbonate solution was treated with lime water. The solubility of  $\text{CaCO}_3$  in water is influenced by

the presence of other substances. Salts of ammonia, for example, tend to render it more soluble, while other substances decrease the solubility. We found, for instance, that in water containing 500 parts per million of pure  $\text{CaSO}_4$ , the solubility of  $\text{CaCO}_3$  was 4.5 parts per million at room temperature, while in water containing 1,000 parts per million  $\text{CaSO}_4$ , the solubility of  $\text{CaCO}_3$  was 4.0 parts per million. The effect of the presence of various salts on the solubility of  $\text{CaCO}_3$ , on account of its importance, has been left for subsequent discussion in detail.

#### SOLUBILITY OF MAGNESIUM HYDROXIDE.

When magnesium carbonate is precipitated from aqueous solution with alkaline salts, the product is invariably basic, that is to say, the carbonate contains a certain amount of hydroxide. The exact composition of this precipitate varies according to conditions, temperature being a very important factor. The solubilities of normal magnesium carbonate or basic magnesium carbonate, therefore, cannot be definitely fixed. It is certain, however, that all modifications of basic magnesium carbonate are much more soluble than magnesium hydroxide, and in water softening, sufficient lime water is added to precipitate all the magnesium in the latter form, i. e., as  $\text{MgO}_2\text{H}_2$ .

The values for the solubility of  $\text{MgO}_2\text{H}_2$  as given by most of the early experimenters, quoted by Comey,<sup>1</sup> are so high as to be obviously worthless. The most probable figures, as shown by Table 4, vary from 9 to 20 parts per million:

TABLE 4.

AUTHORITY	DATE	TEMPERATURE	SOLUBILITY OF $\text{MgO}_2\text{H}_2$	
			Parts per Million	Equivalent Alkalinity, i. e., as $\text{CaCO}_3$
Fresenius <sup>11</sup> .....	1847	"Ordinary" and 100° C.	18	31
Bineau <sup>12</sup> .....	1855	"Ordinary"	10-20	17-34
Kohlrausch and Rose <sup>9</sup> .....	1893	18° C.	9	16

In determining the solubility of this salt, the same method was employed as for  $\text{CaCO}_3$ . The best grade of  $\text{MgO}$ , freshly ignited in order to decompose any carbonate present, was used.

The results are shown in the following table:

TABLE 5.

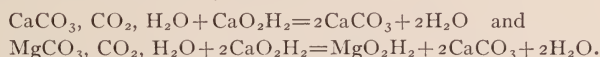
TIME IN HOURS	TEMPERATURE	ALKALINITY IN TERMS OF $\text{CaCO}_3$	MAGNESIUM HYDROXIDE (PARTS PER MILLION)	
			Observed	Corrected for Blank
120.....	22° C.	23	13	12
144.....		23	13	12

Both experiments were conducted in flasks of Jena glass.

This method for  $\text{MgO}_2\text{H}_2$  presents considerable difficulty. Magnesium hydroxide is a colloidal precipitate, which requires a long time to settle, and tends to pass through filter paper. Therefore the results may be somewhat in excess of the true value.

#### PRECIPITATION OF LIME AND MAGNESIA WITH LIME WATER.

Although  $\text{CaCO}_3$  and  $\text{MgCO}_3$  are with difficulty soluble in pure water, they dissolve readily in water containing  $\text{CO}_2$  to the extent of about 1 g. per liter for  $\text{CaCO}_3$ , and 20 g. per liter for  $\text{MgCO}_3$ , at ordinary temperature and pressure. The state in which these salts exist in solution is not definitely established, but they may be represented respectively by the formulas  $\text{CaCO}_3$ ,  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , and  $\text{MgCO}_3$ ,  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ . They are precipitated from solution by lime water according to the following equations:



In order to corroborate the evidence already obtained as to the solubility of  $\text{CaCO}_3$  and  $\text{MgO}_2\text{H}_2$ , and also to throw some light upon the course of the reactions which take place when bicarbonates of lime and magnesia are precipitated with lime water, the following experiments were undertaken.

The solutions used were prepared as follows:

Pure  $\text{CaCO}_3$  was suspended in distilled water, and  $\text{CO}_2$  gas passed in for some time. After the undissolved  $\text{CaCO}_3$  had settled, the supernatant fluid was poured into a large evaporating dish, and allowed to stand over night, thus allowing the excess of free  $\text{CO}_2$  to escape. The liquid was then filtered, giving a fairly strong solution of pure calcium bicarbonate, which could be diluted to any strength desired. Magnesium bicarbonate was prepared in a similar manner from the purest obtainable carbonate of magnesium.

Lime water was made in the usual manner, by shaking distilled water with an excess of freshly slaked lime and filtering the resulting solution. For some experiments a chemically pure lime was used, but the solution obtained from this differed in no appreciable respect, for this purpose, from that obtained with good commercial lime. The strength of these solutions was determined by titration with  $N/50$   $H_2SO_4$ , using phenolphthalein as indicator in the case of lime water, and methyl orange in the case of the bicarbonates. The precipitation experiments were performed as follows: The desired amount of bicarbonate solution was measured with as much accuracy as possible into a bottle of flint glass with a rubber stopper, and brought to the desired temperature. The volumes used varied from 1.8 to 3.5 liters, in bottles of 2 and 4 liters capacity, respectively. 100 c.c. was then quickly withdrawn, and the amount of free  $CO_2$  determined by titration with carefully prepared  $N/22$ ,  $Na_2CO_3$  solution, using phenolphthalein as indicator. Then, the alkalinity and volume of the bicarbonate solution, the amount of free  $CO_2$  gas, and the strength of the lime water being known, a simple calculation sufficed to determine the exact amount of lime water necessary to cause the desired reaction. After the addition of lime water the contents of the bottle were thoroughly mixed by shaking, and maintained at the desired temperature as long as necessary. The amount of calcium carbonate in solution was determined, when desired, by withdrawing a portion of the solution, filtering quickly through paper, and titrating with  $N/50$   $H_2SO_4$  as usual.

#### PRECIPITATION OF CALCIUM CARBONATE.

In the experiments where calcium bicarbonate alone was used, the precipitate which first formed upon adding lime water was colloidal in character, but gradually changed, becoming crystalline in about 20 minutes. This change took place more quickly at higher temperatures than at low temperatures. At  $38^\circ C.$  it required only about 10 minutes, while at  $9^\circ C.$  it required nearly 30 minutes.

Three preliminary experiments with calcium bicarbonate, which were conducted at room temperature as described above, gave the results shown in Table 6. Just enough lime water was added to neutralize all the free  $CO_2$  and precipitate all the calcium as carbonate.

TABLE 6.

Experiment Number	Time of Standing in Hours	Temperature	Parts per Million $CaCO_3$
I. ....	1	$22^\circ C.$	92
II. ....	3.5	22	35
III. ....	6	22	26

It will be noticed that the amount of  $CaCO_3$  remaining in solution was inversely proportional to the time of standing, and it is evident, therefore, that the time element is of much importance in this reaction.



In order to investigate this question more thoroughly, another experiment, like those just described, was carried on at room temperature. Portions of the solution were withdrawn for analysis at intervals, until the amount of  $\text{CaCO}_3$  remaining in solution had reached a minimum.

The results obtained are shown in Table 7.

TABLE 7.

Time in Hours	Temperature	$\text{CaCO}_3$ (Parts per Million)
0.....	.....	180*
1.....	19° C.	70
3.....	20	60
6.....	20	43
24.....	22	22
50.....	22	18
74.....	10	17
122.....	20.5	14.5
218.....	20.5	15
720.....	20.5	17.5

\*Calcium bicarbonate solution before addition of lime water.

In these, as in most of the experiments, the initial alkalinity of the water made no appreciable difference in the rapidity of the reaction after the solution had stood a few hours. In all cases, the results obtained by using phenolphthalein as indicator were one-half of those obtained with methyl orange; and this was found to be true at all stages of the reaction. The experiment recorded in Table 7 was repeated, and figures were obtained which practically checked those given.

The above experiment was then twice repeated, but in one case the solution was kept at a temperature of 2° C., and in the other case at a temperature of 35° C., the other conditions being the same as above. The results of these experiments are given in Tables 8 and 9.

TABLE 8.

Time in Hours	Temperature	Parts per Million of $\text{CaCO}_3$
0.....	1° C.	170*
1.....	2	313
4.....	2	50
6.....	2	40
24.....	2	28
52.....	2	18
77.....	2	17
173.....	2	15.5
312.....	2	15.5

\*Before adding lime water.

TABLE 9.

Time in Hours	Temperature	Parts per Million of $\text{CaCO}_3$
0.....	35° C.	197*
0.25.....	35	43
0.75.....	35	29
5.75.....	35	24
28.0.....	35	18.5
54.0.....	35	16.0
72.0.....	35	18.0

\*Before adding lime water.

These experiments on the precipitation of calcium carbonate with lime water agreed in giving an end point which was practically constant, even though the temperature varied considerably. Thus, at 2° C. the end point showed the solution to contain 15.5 parts per million of calcium carbonate; at 20° it was 14.5; and at 35° it was 16. In these experiments the alkalinity nearly always increased slightly, after attaining a minimum value, but this was evidently due to the action of the solution on the glass of the bottle. It is evident from Tables 8 and 9 that the speed of the reaction, after the first hour, was greater at 35° than at 2°. It was noticed that in the experiment where the water was kept at 2° the calcium carbonate did not come down as a colloidal precipitate, but gradually separated from the solution in very fine crystals.

## PRECIPITATION OF MAGNESIUM HYDROXIDE WITH LIME.

The experiments on the precipitation of magnesium hydroxide with lime were carried on in the same manner as those for calcium carbonate. Two experiments were made at first, one at 20° C. and one at 37° C. The results of these experiments are given in Tables 10 and 11.

TABLE 10.

Time in Hours	Temperature	Observed Alkalinity	Alkalinity Due to $\text{MgO}_2\text{H}_2$
0.....	22° C.	292*	..
0.25.....	21	202	..
0.75.....	21	104	29
9.0.....	20	62	23
27.0.....	20	58	37
99.0.....	20	47	21
195.0.....	20	32	17
267.0.....	20	33	17

\*Before adding lime water.

TABLE 11.

Time in Hours	Temperature	Observed Alkalinity	Alkalinity Due to $\text{MgO}_2\text{H}_2$
0.....	31° C.	220	..
1.5.....	31	73	45.0
5.0.....	34	56	32.0
29.0.....	37	45.5	27.5
53.0.....	37	34.5	17.5
101.0.....	37	29.0	15.0
207.0.....	37	28.0	15.0

In these experiments the observed alkalinities represent, of course, not only the magnesium hydroxide, but also the calcium carbonate derived from the lime water added. In order to obtain figures for the magnesium hydroxide alone, there were subtracted from the observed alkalinities the amounts of normal calcium carbonate present in solution at corresponding times and temperatures, as shown by those experiments in which  $\text{CaCO}_3$  alone was precipitated. These results are given in the last columns of these two tables.

It will be seen from these experiments that the reaction with lime water proceeds more rapidly in the case of magnesium bicarbonate than in the case of calcium bicarbonate.

In order to obtain better values for  $\text{MgO}_2\text{H}_2$ , two precipitation experiments were made together, under the same conditions of temperature and time, one with calcium bicarbonate, the other with magnesium bicarbonate, the temperature being about 35° C. The results of this experiment are given in Table 12.

TABLE 12.

TIME IN HOURS	ALKALINITY IN PARTS PER MILLION		
	Calcium Carbonate	Magnesium Hydrate and Calcium Carbonate	Difference ( $\text{MgO}_2\text{H}_2$ )
0.25.....	55	65	30
0.5.....	40	66	26
1.....	33	60	27
2.....	29	55	26
3.....	28	53	25
5.....	25	50	25
10.....	22	48	26
24.....	18.5	39	20.5
48.....	16	36	20

The reason why the magnesium reaction takes place more rapidly than the calcium may be due to the fact that in the former case the

precipitate is colloidal in character, while in the latter case it is crystalline, although colloidal for a short time at first. It apparently takes some time for calcium carbonate to precipitate completely in this crystalline form.

These facts have an important bearing upon the practical operation of water-softening plants. Since the reaction of lime water on the bicarbonates of calcium and magnesium is not instantaneous, but requires a certain amount of time for completion, it follows that, in order to obtain the best results, water-softening plants must be designed of such capacity as to give time for this reaction to be pretty well advanced before the water leaves the settling tanks. A study of the practical operation of a number of plants has shown that, where the capacities of the coagulation basins are large, the results are better than where they are small. Thus, at Winnipeg, Manitoba, where the time interval is less than two hours, the resulting alkalinity of the water is 80 parts per million, while at Oberlin, Ohio, where the time interval is more than one day, the resulting alkalinity is sometimes as low as 28 parts per million. In certain plants where the time interval is six hours, the results are intermediate between those mentioned.

It is a well-known fact that in most water-softening plants where recarbonization is not practiced, "after-deposits" of lime are apt to form on the sides of the settling tanks, on the sand grains of the filter, and in the distribution pipes. Analyses of these deposits made by the writers have shown them to be composed almost entirely of calcium carbonate, with almost no magnesium. The reason for this appears to be connected with the fact that the rate of the reaction in the case of calcium is slower than in the case of magnesium, and partly, of course, to the fact that the calcium content of most waters considerably exceeds the magnesium content. In one case recently called to our attention, the sand in the filter had become so encrusted with calcium carbonate that its effective size had increased from .40 to .60 mm. These "after-deposits" may be prevented by resorting to the method of recarbonization, which supplies to the water enough free carbonic acid to redissolve, as bicarbonates, the excess of calcium carbonate, and perhaps magnesium hydroxide, which would otherwise gradually settle out.



## SUMMARY AND CONCLUSIONS.

1. The solubility of normal calcium carbonate, as determined by direct experiment, was found to vary from 12 to 14 parts per million; by the precipitation experiments with lime water it was found to be about 14.5 parts per million; but this figure is subject to a subtractive correction on account of the action of the alkaline solution on the glass. The most probable value for the solubility of this salt, at ordinary temperatures, may be taken, therefore, as 13 parts per million.

2. The solubility of magnesium hydrate, as determined by direct experiment, was found to be 12 parts per million. By the precipitation experiments with lime it was found to be 10 parts per million, which is probably more nearly correct, and which may be taken as the most probable value. This is equivalent to an alkalinity of 17 parts per million.

3. The reaction between lime water and calcium bicarbonate requires several days for its completion at ordinary temperatures, but is much more rapid at 37° C. than at the freezing point. The greater part of the precipitate, however, separates within six hours at ordinary temperatures. The longer the period which can be allowed for the reaction, the better, therefore, is the result.

4. The reaction between lime water and magnesium bicarbonate takes place somewhat more rapidly than in the case of calcium. This is apparently due to the fact that the precipitate formed is colloidal instead of crystalline.

The delayed reaction in the case of calcium explains why "after-deposits," so often found in water-softening plants, contain so little magnesium.

5. In order to prevent the formation of "after-deposits" some method of recarbonization is necessary when the time allowed for the reactions is short.

6. The effect of the presence of different salts on the solubility of calcium carbonate and magnesium hydroxide was not determined in these experiments, but left for more complete study.

## APPENDIX.

1. A. M. COMEX. *A Dictionary of Chemical Solubilities*, 1896.

2. CHEVALET. *Ztschr. f. anal. Chem.*, 1869, 8, p. 91.

200 c.c. of  $\text{CaCO}_3$  solution were distilled with five grains  $\text{NH}_4\text{Cl}$ . The first 100 c.c. of the distillate were passed into 10 c.c. dilute  $\text{H}_2\text{SO}_4$ . The  $\text{CO}_2$  was boiled off and the still uncombined acid determined volumetrically. No details are given as to method of making  $\text{CaCO}_3$  solution.

3. HOFFMANN. *Ztschr. f. anal. Chem.*, 1865, 4, p. 414.

A solution of calcium bicarbonate was subjected to prolonged boiling and the filtrate analyzed. It is not stated whether the boiling was conducted in glass, porcelain or platinum.

4. PELIGOT, mentioned by Bineau (*vide sq.*) as having obtained a value of 20 parts per million. No reference given.

5. WELTZIEN. *Ann. d. Chem. u. Pharm.*, 1865, 136, p. 165. No particulars of method given.

6. BINEAU. *Ann. de chim. et phys.*, Par., 1857 (3), 51, p. 290.

Solutions of  $\text{CaCO}_3$  were obtained, (1) by shaking an excess of  $\text{CaCO}_3$  with pure water, (2) by adding a slight excess of calcium salt to dilute solution of  $\text{Na}_2\text{CO}_3$ , and (3) by prolonged boiling of calcium bicarbonate solution. The amount of  $\text{CaCO}_3$  in solution was determined by the addition of an excess of dilute  $\text{H}_2\text{SO}_4$ , expelling  $\text{CO}_2$  by heat or in a partial vacuum, and titrating back with standardized lime water. Special precautions were taken against the action of the  $\text{CO}_2$  of the atmosphere, and the action of  $\text{CaCO}_3$  solution on glass. "The result in which the author places most confidence is 16 parts per million and the solubility of  $\text{CaCO}_3$  is certainly not more than 20 parts per million." These experiments were evidently made with great care.

7. SCHLÖSING. *Compt. rend. Acad. d. Sc.*, Par., 1872, 74, 1552.

"The solubility of normal  $\text{CaCO}_3$  in pure water was determined, with all necessary precautions, at  $16^\circ \text{C}$ ."

8. HOLLEMAN. *Ztschr. f. physik. Chem.*, 1893, 12, 125.

Carefully prepared  $\text{CaCO}_3$  was agitated with water of great purity in small vessels, protected from the  $\text{CO}_2$  of the air by tubes of soda lime. The electrical conductivity of the solution thus obtained was carefully measured, and the amount of salt in solution calculated from this figure. The method was first checked by solutions of known composition, and found to be accurate within about 1 per cent, the greatest deviation being 2.8 per cent.

9. KOHLRAUSCH AND ROSE. *Ztschr. f. physik. Chem.*, 1893, 12, p. 241.

Pure  $\text{CaCO}_3$  was added to water of great purity, in flasks, well shaken, and the electrical conductivity determined until it reached a constant value, from which the solubility of  $\text{CaCO}_3$  could be calculated. The solution was protected from the  $\text{CO}_2$  of the air and special attention was paid to the quality of the glass used. An exactly similar method was used to determine the solubility of  $\text{MgO}_2\text{H}_2$ .

... Kohlrausch and Rose adduce the following advantages for the electrical conductivity method of determining solubilities, which they claim is the best method for this purpose, when the values to be determined are small:

The progress of solution can be followed, and when a constant value is attained, the fact is immediately recognized.

There is no necessity for separating from the solution the excess of undissolved

substance, since the latter introduces no perceptible error in the determination. Hence, filtration as well as evaporation and other lengthy operations, which involve the possible introduction of impurities, are avoided, and the substance can be used in as finely divided condition as may be desired.

Only a small amount of substance is required; the determination requires but a short time, thus avoiding errors introduced by the action of the solvent upon the containing vessel.

The determination is conducted in closed vessels, thus avoiding the influence of foreign matter, for instance, the  $\text{CO}_2$  of the air.

10. KIMBERLY. *Jour. Infect. Dis.*, 1905, Supplm. No. 1., p. 157.

11. FRESNIUS. *Ann. d. Chem. u. Pharm.*, 1847, 59, p. 117.

Carefully purified  $\text{MgO}$  was digested with pure cold water, filtered, and the filtrate evaporated to dryness in platinum dish. No mention is made of precautions taken against action of  $\text{CO}_2$  of the air or the action of  $\text{MgO}_2\text{H}_2$  solution on glass.

12. BINEAU. *Compt. rend. Acad. d. Sc.*, Par., 1855, 41, 510.

Method not given. Author states that solubility is much increased by action of  $\text{CO}_2$  of air.

## EXPERIENCE WITH THE USE OF A NONBASIC ALUM IN CONNECTION WITH MECHANICAL FILTRATION.

GEORGE C. WHIPPLE AND FRANCIS F. LONGLEY.

THE chemical which is used most commonly for the coagulation of waters, in connection with the mechanical system of filtration, is basic sulphate of alumina, more often referred to as "sulphate of alumina," or merely as "alum." In a case where so much depends upon the proper chemical treatment of the water, it is evident that the quality of the chemical used is a matter of fundamental importance. Most of the products which have been furnished for this purpose have been reasonably satisfactory in this respect. Recently, however, an instance has occurred where inferiority in the quality of the alum has caused a noticeable diminution in the efficiency of the filter. The writers have thought that a brief description of this experience might be of interest to others.

Theoretical sulphate of alumina has the formula  $\text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O}$ ; that is, it contains 48.64 per cent of water, 15.32 per cent of alumina ( $\text{Al}_2\text{O}_3$ ), and 36.04 per cent of sulphuric acid ( $\text{SO}_3$ ). The aluminum sulphate of commerce, however, differs somewhat from these theoretical proportions. It usually contains somewhat less water of crystallization and more sulphuric acid and alumina. The excess of alumina over the theoretical amount is generally greater than that of the acid, in the case of alums which have been furnished for filtration purposes. This is what has given rise to the trade name "basic sulphate of alumina." The excess of alumina ( $\text{Al}_2\text{O}_3$ ) over the theoretical amount necessary to combine with the acid ( $\text{SO}_3$ ) usually varies from 1 to 5 per cent, although it sometimes is as high as 10 per cent. The ratio of alumina ( $\text{Al}_2\text{O}_3$ ) actually present in an alum to the amount theoretically necessary to combine with the acid ( $\text{SO}_3$ ) present has been sometimes termed the ratio of basicity. While in the case of most alums which have been furnished for filtration purposes the basicity ratio has exceeded unity, alums are sometimes manufactured for other purposes which have a basicity



ratio less than unity or which are exactly neutral. Neutral or acid alums are sometimes preferred in connection with paper-making, bleaching, etc. It was the use of a neutral alum in connection with mechanical filtration that gave rise to the investigations here recorded.

The sulphate of alumina of commerce is seldom absolutely pure. It usually contains a certain amount of matter which is insoluble in water, small amounts of ferrous and ferric iron, and, occasionally, traces of lime, etc.

Specifications under which alum is obtained for filter purposes usually require that the amount of insoluble matter, the amount of iron, etc., shall not exceed certain fixed quantities. They also provide that the amount of available alumina ( $\text{Al}_2\text{O}_3$ ) shall not be less than a certain specified figure. Seldom or never, however, has it been specified that there shall be an excess of basic alumina, that is, that the alum shall contain more alumina than is theoretically necessary to combine with the sulphuric acid present. Our experience, however, has led us to believe that this is an important point.

A few months ago a mechanical filter was put into operation, and from the start it gave lower bacterial efficiencies than it should have done, considering the character of the plant. The percentage removal of bacteria was between 90 and 95 per cent, instead of 98 per cent, as was expected. This condition of affairs went on for some time, during which the plant was examined in every part, and everything found to be working satisfactorily and in accordance with the most improved regulation. The character of the sand, the method of washing, the rate of wash water, the application of air, the time of coagulation, etc., were all found to be as they should be, yet the results remained unsatisfactory. After a somewhat long series of experiments it was finally determined, by process of exclusion, that the trouble must lie with the alum. In the meantime it had already been noticed that the alum furnished had a low basicity ratio or was practically neutral. An occasional lot was even found to be slightly acid instead of basic. There being no experience on record with the use of such an alum, laboratory experiments were made to determine the difference between a neutral alum and a thoroughly basic alum in the coagulation of water. Several samples were obtained which differed in their basicity from 1.00 to 1.06, and these alums were

added to various samples of water in various amounts from one-half to three grains per gallon. The results were striking. In the case of the basic alums the flocs of coagulant formed more rapidly and were much larger than in the case of the neutral alums. This was found to be true with a clear hard water, with a soft water moderately clear, and with a highly colored water. The neutral alum was then rendered basic by the addition of sodium hydrate, and when this was compared with the same alum without this addition, the flocs were decidedly larger in the case of the basicified sample. The reason for this action is not apparent, but the fact appears to be beyond dispute. It was interesting to note that the application of sodium carbonate to the water before adding the alum did not have the same effect as the addition of sodium hydrate to the alum itself. These experiments were then repeated on a large scale in the actual operation of the filter. Caustic soda was purchased and added to the alum solution in the tanks, but this did not appear to give any improvement in the results of filtration, while it did give rise to a noticeable sludge in the bottom of the alum tanks. It was evident that this method of application was not the proper one, for, as shown by laboratory experiment, although the sodium hydrate dissolved with reasonable care, especially on being warmed, it precipitated within a short time, and the solution apparently lost its basic property. Accordingly the soda solution was made up separately from the alum, and an arrangement was made whereby it was applied to the alum solution just before the latter was added to the water. This method of application appeared to accomplish its purpose, and there was an immediate improvement in the efficiency of the plant, as shown by the following figures; the first line represents the period during which the neutral alum was used without soda; the second with soda:

TABLE 1.

BACTERIA PER C.C.			COLOR		POUNDS PER MILLION GALLONS	
Raw Water	Filtered Water	Per Cent Reduction	Raw Water	Filtered Water	Quantity of Alum	Quantity of Soda
4,370	391	91.0	45	13	267	0
2,520	122	95.2	45	6	237	5

Not only were the bacterial efficiency and the color of the effluent improved, but it was found that equally as good results could be obtained by the use of less alum when the soda was used as when soda was not used. These experiments were so convincing that it was decided to purchase a new lot of alum which had a basicity ratio of at least 1.05. The results obtained with this new lot of alum showed a great improvement over previous ones, a fact made evident by the figures given below. In order to make a further test of the advantages of the basic alum, the use of the two alums was alternated from time to time in order to obtain comparative results.

TABLE 2.

DATES	AVERAGE NO. BACTERIA PER C.C.			QUANTITY ALUM*	ALUM
	Raw Water	Filtered Water	Per Cent Reduction		
Mar. 27-30.....	10,130	500	95.4	190	Neutral alum
Mar. 31-Apr. 4.....	19,755	78	99.4	303	Basic "
April 5-6.....	13,400	152	98.7	204	Neutral "
April 7-8.....	3,810	73	98.2	265	Basic "
April 9-10.....	1,390	74	95.0	278	Neutral "
April 11-20.....	2,212	77	96.7	223	Basic "
Apr. 30-May 8.....	2,314	135	94.5	223	Neutral "
May 9-18.....	990	43	95.8	228	Basic "
May 19-31.....	1,423	99	93.0	260	Neutral "
Average.....	4.022	176	95.5	244	Neutral alum for 30 days
Average.....	4.374	68	98.4	238	Basic alum for 40 days

\*Pounds per million gallons.

The figures show that with the neutral alum the bacterial efficiency for 30 days was 95.5 per cent, while with the basic alum for 40 days it was 98.4, even though the quantity of basic alum used was slightly less than in the case of the neutral alum. Since that time the use of basic alum has been insisted upon, and the efficiency of the filters has been satisfactory.

The following table gives the results of chemical analyses of typical lots of neutral and basic alums which have been used:

TABLE 3.

	Lot No. 3	Lot No. 5	Lot No. 7	Lot No. 9	Lot No. 10	Lot No. 15
Available $\text{Al}_2\text{O}_3$ .....	17.24	17.23	17.18	17.56	17.95	17.57
Total $\text{SO}_3$ .....	40.02	40.70	41.43	40.35	40.17	40.05
Free $\text{SO}_3$ .....	0.20	0.18	0.27	0.23	0.14	0.15
$\text{FeO}$ .....	0.05	0.042	0.05	0.05	0.17	0.20
$\text{Fe}_2\text{O}_3$ .....	0.27	0.28	0.27	0.23	0.14	0.01
Insoluble matter.....	0.00	0.26	0.14	0.00	0.08	0.25
Basicity ratio.....	0.995	1.00	0.982	1.025	1.05	1.03

The neutral alums were found to be unsatisfactory in another respect. When dissolved in the alum tanks the solution was turbid, and when the tank was emptied a considerable amount of sludge was found at the bottom. This insoluble matter caused no little trouble with the small centrifugal pumps which were used in pumping the solution to the tank where its rate of application to the water was controlled. The suspended matter in the alum solution formed deposits in the pipes, which had to be frequently cleaned out in order to maintain a continuously uniform flow. It also necessitated frequent cleaning of the alum tanks. Some of the sediment was due to the presence of ferric iron, but a part of it appeared to be due to undecomposed bauxite used for the manufacture of the alum. The basic alums which have been used have not given nearly as much trouble in this regard as the neutral alums.

The sludge that formed in the bottom of the tank was detrimental, however, only in a mechanical way, that is, by preventing the uniform application of the solution to the water. It did not affect the active strength of the solution (i. e., the available  $\text{Al}_2\text{O}_3$ ), as the following figures will show. Samples of the alum solution were taken from the tanks and allowed to stand in bottles for 24 hours. Then equal portions were taken, of A the clear solution at the surface; and B the solution containing the sludge at the bottom. Gravimetric determinations of the alumina in these samples gave results as follows:

TABLE 4.

	A	B
Sample No. 1.....	0.0078 grams $\text{Al}_2\text{O}_3$	0.0867 grams $\text{Al}_2\text{O}_3$
"    2.....	0.0770 " "	0.0777 " "
"    3.....	0.0809 " "	0.0910 " "

Alums furnished by different dealers vary considerably with respect to their iron contents. In some cases the iron is present chiefly in the ferrous condition, and in other cases in the ferric. It has been the habit of some filtration engineers to specify that the iron shall be present chiefly in the latter form, fearing that in the ferrous condition it would be more likely to unite with the organic matter, pass through the filter, and remain in the filtered water. Our expe-



rience has been that this fear is unfounded, and that there is an advantage in having the iron present in the ferrous form instead of in the ferric. The resulting solution appears to be much clearer, and there is less sludge formed in the tank, less clogging of the pipes, and less interference with the regulation of the alum feed. There is, however, a limit beyond which the amount of iron should not be allowed to pass. Laboratory experiments which we conducted in connection with the matter, as well as experiments made with the entire plant on a larger scale, showed that an amount of ferrous iron equal to 0.5 per cent of the alum gave no trouble. It increased neither the color of the filtered water nor the amount of iron which it contained.

As a result of our experience the following specifications have been drawn up for the purchase of future supplies of alum, and we suggest that specifications for filter alum shall always require that there shall be a substantial excess of alumina, that is, that the alum shall be distinctly basic. This is usually so in any case when the available alumina exceeds 17.5 per cent.

#### SPECIFICATIONS.

The basic sulphate of alumina shall be guaranteed to contain 17 per cent of alumina ( $\text{Al}_2\text{O}_3$ ), soluble in water, and of this amount at least 5 per cent shall be in excess of the amount theoretically necessary to combine with the sulphuric acid present. It shall not contain more than 0.1 per cent of matter insoluble in water and it shall be practically free of chips and debris of all kinds. It shall not contain more than 0.5 per cent of iron ( $\text{Fe}_2\text{O}_3$ ), and the iron shall be preferably in the ferrous state. The alum shall be crushed to pieces of such a size that they will pass through a ring three inches in diameter.

If bidders so desire, they may bid on a product containing a larger amount of soluble alumina, stating, however, the amount which they guarantee. The additional strength will be taken into account in deciding where to place the order

## THE USE OF COPPER SULPHATE IN WATER FILTRATION.

H. W. CLARK AND S. DEM. GAGE.

AN experiment was begun at the Lawrence Experiment Station, during 1904, in order to test the value of copper sulphate as an aid in the purification of polluted water. In this investigation a large experimental filter, 17 feet 4 inches in diameter and containing two and one-half feet in depth of good filter sand, was used. Before the beginning of the copper sulphate experiment, this filter had been in use for 11 years, filtering Merrimac River water. Beginning May 17, 1904, however, copper sulphate was added to the raw water applied to this filter, the amount used at first being one part of the sulphate in 1,000,000 parts of water, and the greatest amount used during the year of experiment being one part in 133,000 parts of water.

The copper sulphate was applied directly to the water upon the surface of the filter, but the volume of water always above the sand allowed a storage of water, after introduction of the sulphate, ranging from five and one-quarter hours to slightly more than eight hours, varying, of course, with the rate of filtration at which the filter was being worked.

Examination of the sand of the filter from time to time, during the experiment and after the experiment had ended, showed the accumulation of a large amount of copper in the sand, and also showed that the copper penetrated throughout the entire depth of the filter. At the end of the experiment, the amount of copper in the surface sand of the filter was 22.8 parts per 100,000; at a depth of 12 inches, 7.6 parts per 100,000; and at a depth of 24 inches, 6.8 parts per 100,000. Giving these results in terms of copper sulphate, the surface sand contained 89.5 parts of copper sulphate per 100,000; at a depth of 12 inches, 79.8 parts; and at a depth of 24 inches, 26.6 parts.

Analyses of the effluent of the filter showed that this effluent con-

tained copper, calculated as copper sulphate, varying in amounts at different times from 1.2 parts per million parts of water to 5.8 parts per million parts of water. At the end of August, 1905, three months after we had ceased to add copper sulphate to the applied water, the effluent contained copper, calculated as copper sulphate, to the amount of 3.7 parts per million parts of water, showing that copper was continually being taken into solution from the deposit within the filter.

The actual volume of water passed through this filter daily varied from 14,000 to 26,000 gallons, and the rate of filtration varied from 2,800,000 gallons per acre daily to 5,200,000 per acre daily.

In connection with this work, experiments were made in regard to the rate of sedimentation of copper sulphate after being mixed with water in large tanks at the experiment station, using, of course, water of the same character as that used in the filtration experiment. In one of these sedimentation experiments it was found that there was practically no sedimentation of the copper until after a period of 20 days; in one, a sedimentation of 50 per cent in 54 days; in one, a sedimentation of about 60 per cent in 62 days; and in one, a sedimentation of 58 per cent in 21 days. No sedimentation occurred in periods of 24 or 48 hours.

If, therefore, in this filtration experiment, a sedimentation tank had been placed between the point of application of the copper sulphate and the filter, a normal period of sedimentation would have effected little copper removal, and practically all the copper not passing through the filter and appearing in the effluent would have collected upon the filtering material.

During the active period of this investigation, samples of the water passing to the filter and the effluent of the filter were taken daily for bacterial examination. The bacterial results obtained from the filter during this experiment, when compared with those obtained during the year previous to the application of copper sulphate to the raw water, show no gain in bacterial removal on account of the use of the copper sulphate—rather the reverse. During the year previous to the use of copper sulphate, the raw water contained 8,300 bacteria per c.c. and the effluent, 73 bacteria per c.c.—or a bacterial efficiency of 99.12 per cent. During the year of copper sulphate

treatment, the raw water contained 7.400 bacteria per c.c., and the effluent of the filter, 114 per c.c.—a bacterial efficiency of 98.5 per cent, 0.62 of 1 per cent less than during the previous year. During both years practically every cubic centimeter sample of the raw water that was tested contained *B. coli*. The effluent of the filter during the year before the copper treatment contained *B. coli* in 13.5 per cent of the cubic centimeter samples examined, and during the year of copper treatment it was found in 26 per cent of the cubic centimeter samples examined.

Summarizing, it can be said that poorer results in water filtration were obtained when using copper sulphate than when operating the same filter without the use of the copper sulphate; a poorer effluent, organically, was obtained, and there was an accumulation of copper upon the sand in the filter that would eventually—if copper, in the form in which it remains upon the sand, has any strength at all as a bactericide—reduce the efficiency of the filter very greatly; that is, the biological actions upon which good results with slow sand filters depend would be badly impaired.



## ON THE BACTERICIDAL ACTION OF COPPER.

H. W. CLARK AND STEPHEN DEM. GAGE.

EARLY in 1904, Moore and Kellerman<sup>1</sup> published the results of their studies of the effect of copper salts on the growth of algæ and bacteria, claiming that minute quantities of copper were sufficient to prevent the development of many troublesome algæ in water supplies, and that a large percentage of the bacteria, including all of the pathogenic bacteria, were destroyed as the result of such treatment. In addition, these observers claimed that storage of a few hours in a copper vessel would effectually free any water from pathogenic bacteria.

Appreciating that a method, apparently so simple, would soon be brought into practical application, and realizing that many factors concerning its safety and efficiency yet remained to be worked out, experiments were begun by the Massachusetts State Board of Health to investigate thoroughly all phases of the subject as applied to the treatment of Massachusetts waters. In the course of this work, much information has been acquired as to the action of copper on the bacterial contents of waters of different kinds, and it is with this phase of the problem that the present paper treats.

The experimental data fall naturally under two heads: (1) experiments in which waters were treated with definite amounts of copper as copper sulphate; and (2) experiments in which the water was placed in contact with metallic copper and allowed to absorb an unknown amount of copper. Each of these two lines of investigation may be subdivided as follows:

- a) Effect on the total numbers of bacteria in the water.
- b) Effect on the numbers of *B. coli* naturally present in polluted waters.
- c) Effect on the numbers of *B. coli* added to water in the form of laboratory cultures.
- d) Effect on the numbers of *B. typhosus* added to water in the form of laboratory cultures.

<sup>1</sup>*U. S. Dept. Agri. Bureau Plant Industry, Bull. No. 64, 1904.*

In addition, experiments have been made to compare the action of other salts frequently used in water purification, such as ferrous sulphate and aluminum sulphate, with copper sulphate, and also to compare the effect of other metals with that of metallic copper.

To the sanitarian the question arises: Does copper in dilute solution destroy the bacteria completely, and do the same laws which apply to the common water bacteria apply equally when dealing with the bacillus of typhoid fever? As we have frequently pointed out, tests made with laboratory cultures of *B. typhosus*, while they have a certain value, are not conclusive evidence as to what would be the behavior of typhoid organisms which have come from fecal matter and have become accustomed to life in water.

The difficulty of isolating typhoid from mixed cultures in water, and the preponderance of other germs in sewage-polluted water, rendered it necessary to attack our problem in a roundabout manner. The method which has given the most reliable information at Lawrence, in investigations of this character, has been to study the relative viability of different laboratory cultures of both *B. typhosus* and *B. coli*, to study the viability of the colon bacillus in a natural state under similar conditions, and from the two sets of experiments to draw inferences as to the viability of the typhoid bacillus in nature under similar conditions.

The weak point in the conclusions of Moore and Kellerman with regard to the destruction of typhoid by copper is that they were drawn from analyses in which the largest amount of water tested was 1 c.c., and the usual amount tested was less than .01 c.c. It is generally conceded, especially when dealing with laboratory cultures, that the great majority of the typhoid bacilli are quickly destroyed by conditions unfavorable to their growth. It has also been repeatedly shown that a few germs are much more resistant than the majority, and may survive even under the most unfavorable conditions for many days. All epidemiological evidence points to the conclusion that the germs which are able to live under unfavorable conditions are also extremely pathogenic, and that, while it may help to destroy the majority of the bacilli, no method of sterilizing water is thoroughly effective unless it will accomplish the destruction of the especially resistant individuals.

It is unsafe to conclude that because a certain species of bacteria, especially a pathogen like *B. typhosus*, is not found in a loopful of the water, or even in 1 c.c., that there is no danger from the use of that water. The average drinking-glass holds about 300 c.c., and until repeated tests of volumes as large as 100 c.c. have been made and the germ proved to be absent, the water under observation cannot safely be said to be free from the test forms.

#### EXPERIMENTAL METHODS AND EXPRESSION OF RESULTS.

In experiments where the water was treated with metallic salts, the water was first drawn in bulk, carefully mixed and sampled, then divided into portions of uniform size, one of which, the control, was carried through untreated, the others being treated with varying amounts of copper sulphate. The containers in all cases were of glass, and the samples were in every case kept in the dark at room temperature. Daily bacterial analyses were made of the contents of the various bottles in each experiment, and in a few experiments a number of analyses were made during the first 24 hours. In every case the contents of the bottles were shaken thoroughly before samples were removed for analysis, thus insuring a fair sample. The volume of water used in the different experiments varied considerably; in some experiments only 100 c.c. of water were placed in each bottle, in others 1,000 c.c. were used, and in a few experiments 3,000 c.c. was the volume treated.

In the experiments in which the waters have been exposed to metallic copper, about 15 liters of water were used in every case, except in the experiments in which a number of metals were under comparison, in which case the volume of water used was about 1,000 c.c.

The containers in the metal experiments have in some cases been of copper, with the control in enameled ware or in glazed stoneware, and in others the waters have been placed in glass and the metals inserted as thin sheets.

In the experiments where the metal was in the form of the container, or was inserted as sheets, it was impossible to shake the contents, and stirring was inadvisable, since it was likely to cause abrasions in the metal and affect the normal rate of solution of the

metal by the water. In these experiments we have resorted to such mixing as could be produced by blowing strongly through the pipettes at the time of sampling. Samples collected under such circumstances are not as representative as would have been the case had a thorough shaking occurred, and some of the variations in such experiments may be attributed to this imperfect sampling.

The determination of the numbers of bacteria and of *B. coli* in raw waters has been made by the regular "Lawrence" methods. In dealing with sterile waters seeded with laboratory cultures of *B. coli* and *B. typhosus*, counts were made on agar plates incubated 18 hours at body temperature, and tests of larger volumes than 1 c.c. were made by mixing broth with the water, incubating at body temperature, and, in case a growth was obtained, by identifying the test organisms by the usual cultural tests. In the following tables the sign + indicates that there was no growth on plates, but that the organism was proved to be present by qualitative tests.

A number of different methods of expressing the copper content of waters have been used in recent publications, the most common being the expression of the ratio of copper sulphate to water by weight. While this method of expression is satisfactory in speaking of treatment with copper sulphate, it is rather out of place in speaking of the amount of copper absorbed in the metallic or colloidal state. As copper sulphate is dissociated in dilute solutions, and as it is the copper *ion* which is apparently the germicide, it is more convenient to express our results in parts of copper per 100,000 parts of water, especially when we wish to compare copper sulphate treatment with the metallic copper treatment. This method of expression has been used throughout in the tables, although in some cases both methods of expression have been used in the text. The following equivalent weights of the metals and of the metallic salts are given for comparison:

Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ): Copper (Cu)=1:0.253

Ferrous sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ): Iron (Fe)=1:0.201

Aluminum sulphate ( $\text{Al}_2(\text{SO}_4)_3$ ): Aluminum (Al)=1:0.158

#### EXPERIMENTS WITH COPPER SULPHATE.

In the following digest of experiments the various bottles in each experiment are designated by the amount of copper added:



*Experiment 159.*—Merrimac River water. Copper range 0.000253 to 253. Duration 10 days. The bacteria in 0.000253 and 0.00253 act like the control. In 0.0253 and 0.253 the bacteria increased. In 2.53 and 25.3 all but a few of the bacteria were killed, these few remaining throughout the experiment. In 253 the bacteria were all destroyed. The bacterial results are shown in Table 1.

TABLE 1.  
MERRIMAC RIVER WATER.  
(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start.....	3,800	.....	.....	.....	.....	..	..	..
1 day.....	.....	10,000	22,400	340,800	30	0	3	0
2 days.....	.....	4,800	16,000	357,000	83,600	0	0	0
3 ".....	.....	2,500	11,800	300,000	103,000	10	6	1
4 ".....	.....	3,600	9,700	315,000	157,500	12	2	0
6 ".....	.....	1,100	2,400	10,300	79,200	27	0	0
8 ".....	.....	1,800	3,500	7,500	97,500	11	2	0
10 ".....	.....	410	870	4,700	113,300	9	8	0

*Experiment 163.*—Merrimac River water containing 1 per cent of sewage Range 0.000253 to 253. Duration 20 days. The numbers of bacteria in the control were higher in 24 hours than they were at the start, but decreased slowly during the experiment. The bacteria in 0.000253, 0.00253, and 0.253 were nearly all destroyed in 24 hours, but the few remaining increased to large numbers in the course of three or four days. In 0.0253 the bacteria increased immediately and then decreased slowly. In 2.53 and 25.3 nearly all of the bacteria were destroyed, but the few remaining were alive during 20 days. In 253 all of the bacteria were destroyed in two days. The bacterial results are shown in Table 2.

TABLE 2.  
MERRIMAC RIVER WATER TO WHICH WAS ADDED 1 PER CENT OF LAWRENCE SEWAGE.  
(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start.....	46,000	.....	.....	.....	.....	..	..	..
1 day.....	69,000	37	8	333,000	80	20	8	3
2 days.....	41,500	10	13	404,500	4,200	6	0	0
3 ".....	12,500	65	34	610,000	3,000	26	23	0
4 ".....	13,700	447,300	125,700	138,500	4,400	28	35	1
6 ".....	7,400	590,000	55	83,100	130,800	14	3	0
8 ".....	8,000	71,000	18,500	220,000	85,200	20	16	0
10 ".....	4,200	37,350	21	56,200	55,500	15	8	0
13 ".....	4,400	25,000	23	33,000	15,600	14	12	0
20 ".....	1,400	100	15	1,700	3,300	7	8	1

*Experiment 210.*—Merrimac River water containing  $\frac{1}{10}$  of 1 per cent of sewage. Duration 131 days. Range 0.000253 to 2.53. The bacteria in the control increased at first and then decreased slowly until the 116th day, when a slight secondary increase was noted. 0.000253, 0.00253, and 0.0253 all increased largely during the first two or

TABLE 3.  
MERRIMAC RIVER WATER TO WHICH WAS ADDED 0.1 PER CENT OF LAWRENCE SEWAGE.  
(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000					
	Control	0.000253	0.00253	0.0253	0.253	2.53
Start.....	17,000	.....	.....	.....	.....	.....
1 day.....	72,500	18,500	115,000	132,000	800	150
2 days.....	64,800	68,400	87,800	87,800	7,000	18
4 ".....	35,000	5,500	24,300	175,000	372,800	65
5 ".....	10,700	12,300	32,600	223,200	458,900	65
6 ".....	17,500	56,500	57,500	162,000	581,400	100
7 ".....	12,000	15,200	50,500	90,000	151,200	100
8 ".....	2,200	4,800	7,300	10,500	75,600	50
11 ".....	11,000	13,300	7,300	35,500	266,300	24
13 ".....	8,000	4,700	4,600	14,000	532,500	25
15 ".....	8,000	7,400	3,000	7,500	450,000	2,600
18 ".....	6,200	5,800	5,100	30,000	770,000	2,400
21 ".....	3,300	2,400	2,650	9,100	320,000	550
25 ".....	600	800	900	5,500	18,400	33
32 ".....	300	450	1,600	4,500	194,400	120
39 ".....	190	135	180	1,700	210,000	30
46 ".....	110	210	205	830	11,400	475
53 ".....	95	475	250	700	10,500	240
60 ".....	65	90	65	300	8,850	75
67 ".....	75	90	160	140	4,500	9
74 ".....	170	85	210	100	1,425	4
81 ".....	21	275	275	24	480	4
88 ".....	140	1,100	725	100	175	35
95 ".....	120	2,700	1,100	530	500	9
103 ".....	38	75	210	55	230	1
109 ".....	55	160	350	300	275	5
116 ".....	1,100	120	5,000	150	325	7
123 ".....	1,100	750	2,350	90	180	27
131 ".....	500	400	1,100	400	550	1

TABLE 4.  
MERRIMAC RIVER WATER TO WHICH WAS ADDED 0.1 PER CENT OF LAWRENCE SEWAGE.  
(B. coli per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000					
	Control	0.000253	0.00253	0.0253	0.253	2.53
Start.....	70	..	..	..	..	..
1 day.....	31	45	32	28	17	10
2 days.....	2	3	2	0	0	0
4 ".....	20	7	10	101	13	23
5 ".....	15	2	17	6	0	0
6 ".....	43	56	48	47	34	42
7 ".....	12	11	0	0	2	0
8 ".....	7	7	8	0	0	50
11 ".....	12	30	5	3	18	0
13 ".....	+	3	0	2	4	3
15 ".....	10	+	0	30	7	6
18 ".....	0	17	27	41	10	16
21 ".....	0	10	11	5	13	13
25 ".....	8	18	25	26	8	11
32 ".....	0	12	16	3	16	6
39 ".....	3	0	10	4	0	1
46 ".....	3	0	3	14	20	14
53 ".....	0	1	0	5	2	1
60 ".....	4	1	0	5	0	8
67 ".....	0	0	7	0	5	3
74 ".....	2	1	0	0	0	1
81 ".....	0	0	0	0	0	0
88 ".....	4	3	2	0	18	5
95 ".....	1	0	0	0	0	1
103 ".....	0	0	0	0	0	1

three days and then decreased slowly. In 0.000253 and 0.00253 a secondary increase in the numbers of bacteria was noted on the 88th and 95th days respectively, but no secondary increase of any importance occurred in 0.0253. In 0.0253 the numbers were considerably reduced in 24 hours, but immediately increased to large numbers on the fifth day, and then slowly declined. In 2.53 over 90 per cent of the bacteria were destroyed in 24 hours, and these numbers remained small and gradually decreased throughout the experiment, with the exception of one small secondary increase which occurred from the 15th to the 18th day.

The *B. coli* in all of the bottles fluctuated considerably, but decreased gradually throughout the experiment, and were found in small numbers up to the 95th day and 103d day, with the exception of 0.253, from which they disappeared on the 67th day. Tests in volumes larger than 1 c.c. were not made. The bacterial results are shown in Table 3, and the *B. coli* results in Table 4.

*Experiment 193.*—Tap water containing 3 per cent of sewage. Duration 187 days. Range 0.0000253 to 25.3. The bacteria in the control increased until the second day and then slowly decreased during 96 days, when a large secondary increase occurred, lasting through the 145th day, when a decrease again started. In 0.0000253, 0.000253, and 0.00253 the bacteria all follow much the same laws as the

TABLE 5.  
LAWRENCE CITY WATER TO WHICH WAS ADDED 3 PER CENT OF LAWRENCE SEWAGE.  
(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.0000253	0.000253	0.00253	0.0253	0.253	2.53	25.3
Start.....	96,400	.....	.....	.....	.....	.....	.....	.....
1/2 hour.....	93,000	76,400	92,600	44,400	51,000	4,800	24,200	4,600
1 ".....	76,000	123,000	92,600	73,200	56,000	2,800	7,700	3,100
2 hours.....	106,500	84,200	110,800	60,200	31,000	1,420	6,200	530
4 ".....	92,300	84,000	132,100	73,000	13,200	350	730	130
6 ".....	84,400	95,000	140,600	84,400	11,000	210	300	80
8 ".....	86,400	75,600	114,500	72,500	8,800	150	175	70
24 ".....	1,000,000	1,120,000	1,150,000	1,150,000	1,500,000	20	2	1
2 days.....	1,100,000	1,400,000	795,200	284,000	1,930,000	220,000	2	0
3 ".....	108,000	370,000	6,400	324,000	1,420,000	990,000	2	3
5 ".....	13,400	44,700	32,200	22,600	1,020,000	1,500,000	5	0
7 ".....	7,500	11,200	16,500	270,000	475,200	276,500	43	5
13 ".....	2,300	4,300	1,000	4,600	4,100	2,360,000	2,400	4
16 ".....	2,400	5,500	5,800	5,400	19,500	73,400	6,600	5
21 ".....	800	4,600	4,800	4,000	11,500	33,800	13,200	1
26 ".....	3,100	4,800	7,200	6,200	12,800	47,000	32,000	5
33 ".....	800	6,500	2,000	3,300	8,500	26,500	15	6
40 ".....	500	6,000	1,250	1,700	5,350	15,500	6	0
47 ".....	3,400	7,700	4,600	2,500	7,600	11,800	1,080	4
55 ".....	480	3,700	2,600	1,200	2,400	162,000	6,800	3
61 ".....	230	185	200	370	440	210,000	4	7
68 ".....	190	650	390	110	850	.....	4	4
75 ".....	225	325	510	160	1,850	150,000	3	3
82 ".....	300	310	550	350	600	88,600	2	0
89 ".....	750	4,700	500	225	275	.....	0	0
96 ".....	300	2,200	250	1,150	510	6,500	0	0
103 ".....	29,100	20,000	740	1,700	660	41,000	0	0
110 ".....	75,000	3,200	1,500	650	270	37,500	0	0
117 ".....	60,000	3,800	475	3,400	300	40,000	0	0
124 ".....	28,600	10,400	590	1,200	240	25,000	0	0
131 ".....	22,600	1,300	880	600	300	7,000	0	0
145 ".....	594,000	5,600	660	725	650	33,500	0	0
160 ".....	650	1,800	1,100	650	57	8,000	0	0
173 ".....	6,000	30,000	3,500	5,300	2,000	47,200	0	0
187 ".....	1,600	2,750	700	300	70	5,900	0	0

control both as to increase, gradual decrease, and a secondary increase after about 100 days. In 0.0253 a primary increase and decrease of bacteria followed the normal curve, only a slight secondary increase being noted. In 0.253 over 99 per cent of the bacteria had disappeared in 24 hours, but these increased to large numbers during the succeeding week and then slowly increased throughout the experiment. In 2.53 the bacteria decreased over 99 per cent in 24 hours, and the numbers remained practically constant until the seventh day, when an increase started which lasted about two weeks, the numbers fluctuating and gradually falling off until the water became sterile on the 89th day. In 25.3 practically all of the bacteria were destroyed in 24 hours, small numbers being occasionally found until the 82d day, when the water became sterile. The bacterial results are shown in Table 5.

*Experiment 176.*—Water from a stagnant reservoir. Duration three days. Range 0.000253 to 0.0253. The bacteria in the control and in 0.000253 decreased, while in 0.00253 and 0.0253 they increased, the greatest increase being noted in the bottle containing the most copper. The bacterial results are shown in Table 6.

TABLE 6.  
WATER FROM STAGNANT RESERVOIR.  
(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000			
	Control	0.000253	0.00253	0.0253
Start.....	120	...	...	...
1 day.....	110	210	400	700
2 days.....	34	55	1,000	19,000
3 ".....	13	31	1,800	12,400

*Experiment 177.*—Water from a stagnant reservoir. Duration 12 days. Range 0.00253 to 0.0253. The bacteria in all of the bottles followed the same law, increasing rapidly during the first two or three days and then decreasing slowly. The bacterial results are shown in Table 7.

TABLE 7.  
WATER FROM STAGNANT RESERVOIR.  
(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000			
	Control	0.00253	0.00506	0.0253
Start.....	3,900	.....	.....	.....
1 day.....	29,400	22,000	24,500	24,500
2 days.....	16,900	22,900	90,200	144,000
3 ".....	10,100	16,400	40,000	165,600
5 ".....	6,800	4,100	21,000	10,500
7 ".....	5,000	4,700	4,000	6,900
9 ".....	4,200	10,500	32,500	90,000
12 ".....	270	420	3,600	2,500

*Experiment 179.*—Water from a stagnant reservoir. Duration eight days. Range 0.00253 to 2.53. The bacteria in the control fluctuated somewhat, but decreased throughout the experiment. In 0.00253 the numbers decreased during the first six



days and then showed an increase on the eighth day. In 0.0253 the numbers decreased during the first two days and then increased largely, the same being true with 0.253. In 2.53 over 90 per cent of the bacteria were destroyed in 24 hours, and continued to decrease to the sixth day, a large secondary increase being noted on the eighth day. The bacterial results are shown in Table 8.

TABLE 8.  
WATER FROM STAGNANT RESERVOIR.  
(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.00253	0.0253	0.253	2.53
Start.....	300	....	.....	.....	.....
1 day.....	35	255	100	135	21
2 days.....	45	400	36	28	9
4 ".....	700	300	113,600	97,200	1
6 ".....	80	75	10,600	1,800	2
8 ".....	245	4,100	52,000	1,900	2,300

*Experiment 187.*—Water from a stagnant reservoir. Duration 12 days. Range 0.00253 to 2.53. The bacteria in the control increased slowly throughout the experiment. In 0.00253 they increased during the first three days, and then fluctuated, but decreased throughout the remainder of the experiment. In 0.0253 over 90 per cent of the bacteria were killed in 24 hours, but the remainder increased rapidly throughout the experiment. In 0.253 the bacteria decreased during the first two days and then increased largely until the fifth day, a decrease following throughout the experiment. In 2.53 over 95 per cent of the bacteria were killed in 24 hours, the rest remaining practically constant, with the usual fluctuations. The bacterial results are shown in Table 9.

TABLE 9.  
WATER FROM STAGNANT RESERVOIR.  
(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.00253	0.0253	0.253	2.53
Start.....	265	.....	.....	.....	..
1 day.....	230	210	16	95	9
2 days.....	1,500	2,500	1,300	65	0
3 ".....	425	11,000	10,400	23,200	2
4 ".....	1,260	720	38,800	442,800	0
5 ".....	1,118	7,200	48,400	630,000	..
7 ".....	4,500	700	1,900	241,000	3
9 ".....	8,000	865	51,000	75,600	5
12 ".....	15,500	3,200	24,000	240,000	8

*Experiment 185.*—Driven well water from Methuen town supply. Duration 133 days. Range 0.000253 to 0.253. There were only 31 bacteria in this water at the start. In all the samples a large increase was noted in 24 to 48 hours. The numbers in the control, 0.000253, and 0.00253 remained practically constant after 48 hours during 45 days, after which they began to decrease slowly. In 0.0253 a considerable decrease

occurred about the 84th day, the numbers remaining low after that time. In 0.253 the numbers were below 100 on the 31st day and continued low, with the exception of one count throughout the experiment. The bacterial results are shown in Table 10.

TABLE 10.  
DRIVEN WELL WATER FROM METHUEN TOWN SUPPLY.  
(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.000253	0.00253	0.0253	0.253
Start.....	31	.....	.....	.....	.....
1 day.....	15,700	6,700	6,300	3,700	300
2 days.....	3,240	275	19,500	27,000	53,500
3 ".....	1,050	440	17,000	30,000	10,300
4 ".....	1,300	315	3,500	66,000	33,600
5 ".....	11,800	14,500	4,700	181,100	55,000
7 ".....	14,000	675	13,500	97,200	21,000
8 ".....	920	9,500	20,000	66,000	41,600
10 ".....	600	440	13,800	9,100	1,400
12 ".....	500	5,600	16,600	46,000	1,200
15 ".....	900	13,500	11,000	80,600	500
17 ".....	7,500	3,600	17,500	33,000	100
19 ".....	1,500	7,200	13,500	10,800	3,100
21 ".....	5,200	4,700	10,600	20,600	260
23 ".....	6,400	1,600	4,700	11,600	125
25 ".....	8,000	9,600	13,200	17,600	230
28 ".....	430	2,900	5,600	14,800	140
31 ".....	4,100	6,500	5,900	13,600	15
35 ".....	4,300	3,100	3,900	14,800	38
38 ".....	2,500	4,600	4,300	12,000	95
42 ".....	335	85	3,600	11,000	65
45 ".....	1,100	1,800	2,400	9,200	4
49 ".....	290	100	600	5,100	1
57 ".....	200	40	1,600	4,000	5
65 ".....	3,200	75	2,000	800	9
70 ".....	2,400	800	700	250	18
77 ".....	1,600	12	55	1,700	340
84 ".....	900	65	550	10	0
91 ".....	1,000	475	2,900	105	10
99 ".....	200	200	350	0	6
105 ".....	95	25	625	33	8
112 ".....	35	24	110	2	2
119 ".....	140	37	375	28	10
126 ".....	133	15	250	8	7
133 ".....	143	330	265	24	19

*Experiment 164.*—48 hour broth culture of *B. coli* diluted 1:10,000 with sterile tap water. Duration six days. Range 0.000253 to 253. The *B. coli* in the control decreased during the first two days and then began to increase. In 0.000253 about

TABLE 11.  
48 HOUR BROTH CULTURE *B. COLI* DILUTED 1:10,000 WITH STERILE TAP WATER.  
(*B. coli* per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start.....	15,400	...	..	..	..	..	..	..
1 day.....	2,500	800	2	1	0	0	0	0
2 days.....	1,200	67	3	0	0	0	0	0
3 ".....	1,600	7	0	0	0	0	0	0
4 ".....	25,600	5	0	0	0	0	0	0
6 ".....	580,000	0	0	0	0	0	0	0

50 per cent were killed in 24 hours, the numbers decreasing rapidly until the water became sterile on the sixth day. In 0.00253 and 0.0253 all but a few of the germs were killed in 24 hours, and the water became sterile on the third and second days, respectively. In 0.253 to 253 inclusive all of the test organisms were killed in 24 hours. The detailed results are shown in Table 11.

*Experiment 161.*—48 hour broth culture of *B. typhosus* diluted 1:10,000 with sterile tap water. Duration three days. Range 0.000253 to 253 inclusive. The test organisms disappeared from the control in 24 hours. In 0.000253 a large increase was noted in 24 hours. In 0.00253 about 90 per cent of the organisms were destroyed in 24 hours, and the water became sterile on the third day. In 0.0253 to 253 inclusive, like the control, the waters were sterile in 24 hours. The detailed results are shown in Table 12.

TABLE 12.

48 HOUR BROTH CULTURE OF *B. TYPHOSUS* DILUTED 1:10,000 WITH STERILE TAP WATER.  
(*B. typhosus* per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start.....	3,100	.....	...	..	..	..	..	..
1 day.....	0	23,300	48	0	0	0	0	0
2 days.....	0	8,400	2	0	0	0	0	0
3 ".....	0	21,000	0	0	0	0	0	0

*Experiment 191.*—48 hour broth culture of *B. typhosus* diluted 1:10,000 with sterile tap water. Duration 28 days. Range 0.000253 to 0.253. The test organisms in the control dropped off gradually from 115,000 at the start to only four organisms

TABLE 13.

24 HOUR BROTH CULTURE OF *B. TYPHOSUS* DILUTED 1:10,000 WITH STERILE TAP WATER.  
(*B. typhosus* per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.000253	0.00253	0.0253	0.253
Start.....	115,500	.....	.....	.....	...
1/2 hour.....	71,000	234,000	14,500	6,500	520
1 ".....	100,800	90,000	105	1,000	24
2 hours.....	47,000	30,000	85	65	26
4 ".....	25,000	4,800	2	18	1
6 ".....	3,000	900	+	2	+
8 ".....	2,800	770	1	+	+
24 ".....	770	5	+	1	+
2 days.....	12	6,300	6,525	+	+
3 ".....	4	240	.....	+	+
5 ".....	52,000	1,100	8	+	+
6 ".....	420,000	22,000	8	+	+
7 ".....	260,000	54,000	8	0 <sup>+</sup>	0 <sup>+</sup>
8 ".....	230,000	47,000	5	0 <sup>+</sup>	0 <sup>+</sup>
9 ".....	220,000	35,000	4	..	..
10 ".....	80,000	30,000	2	..	..
12 ".....	260,000	20,000	7	..	..
14 ".....	118,800	42,000	+	..	..
16 ".....	86,400	36,000	+	..	..
20 ".....	61,200	12,300	+	..	..
22 ".....	72,100	24,200	+	..	..
28 ".....	237,000	16,000	+	..	..

\*1 c.c.=0, 10 c.c.=+.

†1-10 c.c.=0, 100 c.c.=+.

‡1, 10 and 100 c.c.=0.

on the third day, and then increased rapidly to over 400,000 on the sixth day, after which they decreased slowly throughout the experiment. The same phenomenon was noted in 0.000253, the bacteria dropping off to five individuals in 24 hours, after which they increased to over 54,000 on the seventh day, and then gradually decreased. In 0.00253 the organisms were destroyed rapidly, about 90 per cent being killed during the first hour, and in 24 hours it required 10 c.c. of the water to detect the test organism. On the second day, however, the organisms had increased, and small numbers were present until the 12th day. From the 14th to 28th days *B. typhosus* was not found in 1 c.c., but was present in 10 c.c. In 0.0253 over 90 per cent of the organisms were destroyed in 30 minutes, and nearly all had disappeared in six hours. From the second to fifth days the organism was found present in 10 c.c., and on the sixth day it was found present in 100 c.c., the water becoming sterile on the seventh day. In 0.253 over 95 per cent of the organisms disappeared in the first 30 minutes and the decrease was so rapid that after six hours it required 10 c.c. of the water to obtain a positive test. The organism was found continuously in 10 c.c. through the fifth day, and was found in 100 c.c. on the sixth day, the water becoming sterile on the seventh day. The detailed results are shown in Table 13.

*Experiment 194.*—24 hour broth culture of *B. typhosus* diluted 1:500 with sterile tap water. Duration 10 days. Range 0.0000253 to 2.53. In this experiment the typhoid bacilli were added to the water as a broth culture, and the water in the various bottles was allowed to stand four days before adding copper, the idea being that the organisms would have become in a measure inured to their new environment before they were subjected to the action of the copper sulphate. The numbers of *B. typhosus*

TABLE 14.  
24 HOUR BROTH CULTURE OF *B. TYPHOSUS* DILUTED 1:500 WITH STERILE TAP WATER AND ALLOWED  
TO STAND FOUR DAYS BEFORE ADDING COPPER SULPHATE.  
(*B. typhosus* per c.c.)

ELAPSED TIME	BEFORE ADDING COPPER							
	A	B	C	D	E	F	G	H
Start.....	1,280,000	1,200,000	1,200,000	1,200,000	1,200,000	1,280,000	1,280,000	1,280,000
1 day.....	450,000	200,000	120,000	104,400	200,000	420,000	480,000	300,000
2 days.....	1,080,000	79,200	28,500	59,000	93,600	1,090,000	950,000	940,000
3 ".....	2,200,000	210,000	75,600	75,600	165,600	1,850,000	2,430,000	2,200,000
4 ".....	3,000,000	313,200	118,800	85,200	777,600	2,500,000	2,430,000	73,000

COPPER ADDED—PARTS PER 100,000.								
Elapsed Time	Control	Duplicate Control	0.0000253	0.000253	0.00253	0.0253	0.253	2.53
30 minutes.....	.....	.....	149,100	70,000	399,600	2,090,500	1,887,600	34,000
1 hour.....	.....	.....	183,600	239,000	529,100	1,120,000	1,358,500	27,500
2 hours.....	.....	.....	159,800	72,000	626,400	1,657,500	640,000	2,225
4 ".....	.....	.....	175,000	149,000	748,400	4,490,400	323,800	142
6 ".....	.....	.....	205,200	109,400	1,101,600	2,814,000	48,800	15
24 ".....	3,400,000	902,900	604,800	379,000	3,700,000	4,200,000	4	1
2 days.....	2,750,000	2,000,000	1,040,000	1,080,000	2,520,000	2,750,000	0	0
3 ".....	.....	3,000,000	1,120,000	1,200,000	2,200,000	2,450,000	0	0
4 ".....	265,000	4,400,000	330,000	14,400,000	4,500,000	3,000,000	0	0
8 ".....	2,200,000	4,600,000	10,300,000	9,360,000	3,100,000	2,060,000	.....	.....
10 ".....	1,300,000	2,230,000	.....	250,000	1,650,000	1,300,000	.....	.....



during these four days increased in four of the bottles and decreased more or less in the other four. In the two bottles which were retained as controls the *B. typhosus* continued to increase during the 10 days they were under observation, the same being true of the waters containing copper 0.0000253, 0.000253, and 0.00253. In the water containing copper 0.0253 the numbers of *B. typhosus* remained practically constant during the 10 days. In 0.253 and 2.53 the numbers fell off rapidly, being practically all eliminated in 24 hours, and the waters becoming sterile in 48 hours. Tests were not made in volumes larger than 1 c.c. in this experiment. The detailed results are shown in Table 14.

## EXPERIMENTS WITH METALLIC COPPER.

*Experiment 175.*—18.5 liters of Merrimac River water in copper dish. Control in a stone crock. Exposed copper area 3,200 square c.c. Duration 20 days. The bacteria in the control increased. The bacteria in the copper dish, with the exception of one slight increase, fell off gradually.

The *B. coli* in the control were found in 1 c.c. on the eighth day and in 100 c.c. on the 15th day. In the copper dish the *B. coli* disappeared from 100 c.c. on the third day. The bacterial results are shown in Table 15 and the *B. coli* results in Table 16.

*Experiment 178.*—18.5 liters of tap water containing 10 per cent of sewage in a copper dish. Control in a stone crock. Exposed copper area 3,200 square c.c. Duration 76 days. Copper absorbed 0.46 parts per 100,000 (87 days). The bacteria

TABLE 15.  
(Bacteria per c.c.)

ELAPSED TIME	EXPERIMENT 175 MERRIMAC RIVER WATER		EXPERIMENT 178 TAP WATER CONTAINING 10 PER CENT OF SEWAGE	
	Control in Stone Crock	Copper Dish	Control in Stone Crock	Copper Dish
Start.....	1,400	1,400	320,000	390,000
1 day.....	9,300	1,500	1,050,000	1,440,000
2 days.....	11,000	17,300	2,080,000	3,430,000
3 ".....	488,500	337	470,000	2,600,000
4 ".....	1,114,000	1,000	350,000	250,000
6 ".....	704,900	3,100	12,000	420,000
8 ".....	16,200	95	12,000	60,000
10 ".....	107,000	180	1,230,000	210,000
13 ".....	125,000	85	110,000	30,000
15 ".....	307,000	85	73,400	26,500
17 ".....	414,000	30	77,800	39,000
20 ".....	1,490,000	37	300,000	39,000
22 ".....	.....	.....	73,000	20,000
24 ".....	.....	.....	240,000	4,000
28 ".....	.....	.....	180,000	12,300
30 ".....	.....	.....	230,000	5,000
32 ".....	.....	.....	180,000	4,500
34 ".....	.....	.....	118,800	2,800
36 ".....	.....	.....	29,000	3,000
38 ".....	.....	.....	168,500	2,700
41 ".....	.....	.....	55,000	2,500
44 ".....	.....	.....	86,400	2,400
48 ".....	.....	.....	101,500	3,000
55 ".....	.....	.....	174,200	1,100
62 ".....	.....	.....	93,600	200
70 ".....	.....	.....	172,800	100
76 ".....	.....	.....	350,000	3

TABLE 16.  
(B. coli per c.c.)

ELAPSED TIME	EXPERIMENT 175 MERRIMAC RIVER WATER		EXPERIMENT 178 TAP WATER CONTAINING 10 PER CENT OF SEWAGE	
	Control in Stone Crock	Copper Dish	Control in Stone Crock	Copper Dish
Start.....	11	11	2,600	5,000
1 day.....	11	..	750	550
2 days.....	11	o†	700	100
3 ".....	+	o†	100	+
4 ".....	+	o†	+	+
6 ".....	+	o†	6	+
8 ".....	5	o†	+	3
10 ".....	+*	o†	1	6
13 ".....	+*	..	2	+
15 ".....	+*	..	o†	4
17 ".....	o†	..	o†	3
20 ".....	o†	..	o†	4
22 ".....	..	..	o†	o†
24 ".....	..	..	o†	o†

\*1 c.c.=0, 100 c.c.=+.

†100 c.c.=0.

in the control were practically constant throughout. The bacteria in the copper dish increased rapidly and then slowly decreased until the water was practically sterile on the 76th day.

The B. coli decreased rapidly in both cultures. In the control it was found in 1 c.c. on the 13th day, but disappeared from 100 c.c. on the 15th day. In the copper, B. coli were found in small numbers on the 20th day, five days after they had completely disappeared from the control. The bacterial results are shown in Table 15 and the B. coli results in Table 16.

*Experiment 183.*—18.5 liters of Merrimac River water in a copper dish, with control in an enameled dish. Exposed copper area 2,900 square c.c. Duration 71 days. Copper absorbed 0.30 parts per 100,000. Bacteria drop off gradually in both copper and control, both becoming practically sterile at end of experiment.

B. coli were found in 1 c.c. of the control on the 17th day and in 100 c.c. on the 28th day, disappearing on the 30th day. In the copper, B. coli were found in small numbers up to the sixth day, but disappeared from 100 c.c. on the eighth day. The bacterial results are shown in Table 17 and the B. coli results in Table 18.

*Experiment 200.*—17.3 liters of Merrimac River water in a copper dish with control in an enameled dish. Exposed copper surface 2,900 square c.c. Duration 64 days. Copper absorbed 1.0 part per 100,000 (78 days). Bacteria in both control and copper dish decrease gradually, the decline in the copper dish being more rapid. The contents of the copper dish were sterile on the 55th day, and in the control only a few bacteria remained alive.

B. coli were found in the control in 1 c.c. as late as the 20th day, but disappeared from 100 c.c. on the 28th day. In the copper, B. coli disappeared from 1 c.c. on the fourth day, but were found in 100 c.c. as late as the 34th day, six days after they had disappeared from the control. The bacterial results are shown in Table 17 and the B. coli results in Table 18.

TABLE 17.  
(Bacteria per c.c.)

ELAPSED TIME	EXPERIMENT 183		EXPERIMENT 200	
	MERRIMAC RIVER WATER		MERRIMAC RIVER WATER	
	Control in Enameled Dish	Copper Dish	Control in Enameled Dish	Copper Dish
Start.....	600	1,200	11,000	10,500
$\frac{1}{2}$ hour.....	....	....	12,300	16,500
1 ".....	....	....	11,200	11,500
2 hours.....	800	500	10,800	7,300
4 ".....	700	400	11,000	7,200
6 ".....	400	135	12,000	6,000
8 ".....	800	200	20,000	3,800
24 ".....	1,300	65	16,000	104
2 days.....	1,044	120	6,500	400
3 ".....	7,700	....	12,000	200
4 ".....	103	763	1,200	42
6 ".....	164	133	5,500	133
8 ".....	245	156	5,400	40
10 ".....	250	110	12,800	15
13 ".....	275	25	300	7
15 ".....	180	12	....	....
17 ".....	792	13	....	....
20 ".....	20	10	1,200	15
23 ".....	2,500	9	....	....
28 ".....	100	13	46	9
30 ".....	260	13	....	....
34 ".....	82	10	28	2
42 ".....	90	12	11	0
48 ".....	10	6	65	3
55 ".....	3	1	6	0
64 ".....	3	4	2	0
71 ".....	5	6	....	....

TABLE 18.  
(B. coli per c.c.)

ELAPSED TIME	EXPERIMENT 183		EXPERIMENT 200	
	MERRIMAC RIVER WATER		MERRIMAC RIVER WATER	
	Control in Enameled Dish	Copper Dish	Control in Enameled Dish	Copper Dish
Start.....	32	44	...	...
$\frac{1}{2}$ hour.....	..	..	01	146
1 ".....	..	..	118	114
2 hours.....	48	10	118	97
4 ".....	23	12	120	96
6 ".....	48	+	74	63
8 ".....	44	12	105	15
24 ".....	30	+	52	+
2 days.....	0	3	8	+
3 ".....	25	4	8	11
4 ".....	7	+	+	+
6 ".....	1	10	11	+
8 ".....	11	o†	+	+
10 ".....	7	o†	+	+
13 ".....	1	o†	+	+
15 ".....	+	o†	..	..
17 ".....	+	..	..	..
20 ".....	+	..	6	+
23 ".....	+	..	..	..
28 ".....	+	..	o†	+
30 ".....	o†	..	..	..
34 ".....	o†	..	o†	+
42 ".....	..	..	o†	o†
48 ".....	..	..	0	o†

\*1 c.c.=0, 100 c.c.=+.

†100 c.c.=0.

*Experiment 182.*—Three glass vessels containing respectively 18.9 liters of Merrimac River water, effluent from a sewage filter, and driven well water. 2,200 square c.c. of copper surface were inserted in each in the form of thin sheets. Duration seven days. Copper absorbed by river water 0.062, by sewage effluent 1.822, by driven well water 0.035. The bacteria in all samples increased. The bacterial results are shown in Table 19.

*Experiment 186.*—Duplicate of Experiment 182. Duration 14 days. Copper absorbed by river water 0.082, by sewage effluent 2.400, by well water 0.047. The numbers of bacteria in the river water showed only small fluctuations. In the sewage effluent the bacteria decreased about 85 per cent until the fifth day, and then increased until the 13th day. The bacteria in the well water increased to a maximum on the 10th day and then slowly decreased. The bacterial results are shown in Table 19.

TABLE 19.  
WATER IN GLASS VESSELS CONTAINING SHEETS OF METALLIC COPPER.  
(Bacteria per c.c.)

ELAPSED TIME	EXPERIMENT 182			EXPERIMENT 186		
	Merrimac River Water	Effluent Sewage Filter	Driven Well Water	Merrimac River Water	Effluent Sewage Filter	Driven Well Water
Start.....	1,600	14,500	900	2,400	2,000	195
1 day.....	1,500	972	443	525	245	375
2 days.....	1,600	4,300	3,400	545	345	560
3 ".....	2,000	1,100	12,300	340	240	40,000
4 ".....	7,500	32,500	6,175	3,500	370	87,300
5 ".....	21,000	142,000	30,000	655	1,800	78,100
6 ".....	4,400	22,000	5,110	240	1,420	78,100
7 ".....	5,000	10,800	21,600	720	1,800	60,000
8 ".....	.....	.....	.....	900	11,200	43,000
9 ".....	.....	.....	.....	700	6,875	66,600
10 ".....	.....	.....	.....	6,000	12,000	100,000
11 ".....	.....	.....	.....	2,400	8,000	73,400
12 ".....	.....	.....	.....	585	63,000	70,000
13 ".....	.....	.....	.....	575	60,000	72,000
14 ".....	.....	.....	.....	3,200	4,750	69,000

*Experiment 184.*—48 hour broth culture of *B. coli* diluted 1:10,000 with sterile tap water in a copper dish, with control in an enameled dish. 18.5 liters of water. Copper surface 2,900 square c.c. Duration six days. Copper absorbed 0.50 parts per 100,000 (sterilized in dish and stood 12 days). *B. coli* in the control show a normal increase. In the copper over 99 per cent died out in 24 hours, but a few were alive on the sixth day. The detailed results are shown in Table 20.

*Experiment 189.*—48 hour broth culture of *B. coli* diluted 1:10,000 with sterile tap water in a copper dish, with a control in an enameled dish. 18.5 liters of water. Copper surface 2,900 square c.c. Duration 12 days. Copper absorbed 0.68 parts per 100,000 (21 days). *B. coli* in control show a normal increase. In copper the numbers remained practically constant during five days, the test organisms being found in one c.c. on the eighth day, but having disappeared from that volume on the 10th day. The detailed results are shown in Table 20.



TABLE 20.

48 HOUR BROTH CULTURE OF *B. COLI* DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. coli per c.c.)

ELAPSED TIME	EXPERIMENT 184		EXPERIMENT 189	
	Control in Enameled Dish	Copper Dish	Control in Enameled Dish	Copper Dish
Start.....	370,000	350,000	430,000	360,000
2 hours.....	750,000	550,000	700,000	420,000
4 ".....	320,000	450,000	118,800	98,000
6 ".....	340,000	500,000	275,300	110,200
8 ".....	440,000	159,800	260,000	350,000
24 ".....	750,000	735	21,000	9,500
2 days.....	390,000	2	77,800	190,800
3 ".....	4,300,000	1	479,300	2,100,000
4 ".....	.....	.....	2,851,200	180,000
5 ".....	1,570,000	+	3,800,000	539,600
6 ".....	4,700,000	+	1,430,000	400
8 ".....	.....	.....	392,100	1
10 ".....	.....	.....	2,750,000	0
12 ".....	.....	.....	450,000	0

*Experiment 199.*—48 hour broth culture of *B. coli* diluted 1:10,000 with sterile tap water in a copper dish, with control in an enameled dish. 19 liters of water. Copper surface 3,050 square c.c. Duration 62 days. Copper absorbed 0.54 parts per 100,000 (72 days). The numbers of *B. coli* in the control remained practically unchanged during 62 days. In the copper over 99 per cent of the organisms were killed in 24 hours, and it required 10 c.c. to give a positive for the organisms on the second and third days. On the fourth day a secondary increase commenced, and

TABLE 21.

48 HOUR BROTH CULTURE OF *B. COLI* DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. coli per c.c.)

Elapsed Time	Control in Enameled Dish	Copper Dish
Start.....	280,000	440,000
½ hour.....	280,000	380,000
1 ".....	380,000	290,000
2 hours.....	360,000	300,000
4 ".....	240,000	78,400
6 ".....	201,600	68,400
8 ".....	147,600	10,500
24 ".....	111,600	15
2 days.....	146,900	+
3 ".....	1,026,000	+
4 ".....	852,000	22
6 ".....	630,000	1
8 ".....	410,000	4,000
10 ".....	1,560,000	750
13 ".....	1,000,000	+
20 ".....	170,000	+
27 ".....	46,100	+
33 ".....	220,000	+
40 ".....	990,000	+
47 ".....	400,000	0
54 ".....	520,000	0
62 ".....	100,000	0

\*1 c.c.=0, 10 c.c.=+.

†1 and 10 c.c.=0, 100 c.c.=+.

‡1, 10, and 100 c.c.=0.

on the eighth day the number of *B. coli* had increased to 4,900. On the 10th day a decline was noted. From the 13th to the 27th day the organism was found in 10 c.c., but disappeared from 100 c.c. on the 33d day. The detailed results are shown in Table 21.

*Experiment 190.*—48 hour broth culture of *B. typhosus* diluted 1:10,000 with sterile tap water in a copper dish, with control in an enameled dish. 18.5 liters of water. Copper surface, 2,900 square c.c. Duration three days. The numbers of *B. typhosus* in the control decreased rapidly, over 99 per cent having disappeared in three days. In the copper the same rapid decrease was noted, over 99 per cent of the organisms having disappeared in six hours, and practically all in eight hours. After 24 hours the organism was not found in 1 c.c., but was detected in 100 c.c., and on the second day it had disappeared from 100 c.c. The detailed results are shown in Table 22.

TABLE 22.

48 HOUR BROTH CULTURE OF *B. TYPHOSUS* DILUTED 1:10,000 WITH STERILE TAP WATER  
(*B. typhosus* per c.c.)

Elapsed Time	Control in Enameled Dish	Copper Dish
Start.....	180,000	270,000
$\frac{1}{2}$ hour.....	200,000	93,600
1 ".....	47,000	39,000
2 hours.....	26,500	14,500
4 ".....	300	700
6 ".....	2,000	200
8 ".....	1,800	+
24 ".....	2,000	+*
2 days.....	330	0†
3 ".....	110	0†

\*1-10-100 c.c.=0.

†1-10 c.c.=0, 100 c.c.=+.

#### COMPARISON OF COPPER AND COPPER SULPHATE WITH OTHER METALS AND SALTS.

In the following digest of experiments, the various bottles of experiments with ferrous sulphate and aluminum sulphate, are designated by the amount of iron or aluminum present, in parts per 100,000.

*Experiment 210.*—Merrimac River water treated with ferrous sulphate. Range 0.002 to 2.01. Duration 131 days. The bacteria in the control increased during the first 24 hours and then gradually decreased until the 116th day, when a small secondary increase occurred. In .002 the increase in 24 hours was larger than in the control, after which the numbers decreased steadily throughout the period of observation. In 2.01 over 95 per cent of the bacteria were destroyed in 24 hours, but those remaining were able to increase steadily until the sixth day, after which a decline set in, lasting till the 109th day. A slight secondary increase began about the 116th day.

*B. coli* were found in gradually decreasing numbers in the control until the 95th day, and in .002 until the 88th day. In 2.01 the *B. coli* were nearly all destroyed in

TABLE 23.  
MERRIMAC RIVER WATER TO WHICH WAS ADDED 0.1 PER CENT OF LAWRENCE SEWAGE.  
(Bacteria per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000		
	Control	0.002	2.01
Start.....	17,000	.....	.....
1 day.....	72,500	123,500	500
2 days.....	64,800	86,400	1,300
4 ".....	35,000	13,300	8,500
5 ".....	10,700	10,500	7,300
6 ".....	17,500	36,000	83,000
7 ".....	12,000	12,500	6,550
8 ".....	2,200	61,400	.....
11 ".....	11,000	10,800	26,500
13 ".....	8,000	1,200	9,500
15 ".....	8,000	3,700	9,500
18 ".....	6,200	5,000	7,400
21 ".....	3,300	900	2,200
25 ".....	600	1,600	1,440
32 ".....	390	110	350
39 ".....	190	205	410
46 ".....	110	245	305
53 ".....	95	90	260
60 ".....	65	90	95
67 ".....	75	110	46
74 ".....	170	75	250
81 ".....	21	57	28
88 ".....	140	110	85
95 ".....	120	12	67
103 ".....	38	8	18
109 ".....	55	100	80
116 ".....	1,100	180	700
123 ".....	1,100	375	275
131 ".....	500	170	1,200

TABBE 24.  
MERRIMAC RIVER WATER TO WHICH WAS ADDED 0.1 PER CENT OF LAWRENCE SEWAGE.  
(B. coli per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000		
	Control	0.002	2.01
Start.....	70	..	..
1 day.....	31	20	+
2 days.....	2	1	+
4 ".....	20	+	+
5 ".....	15	+	1
6 ".....	43	58	+
7 ".....	12	+	+
8 ".....	7	+	+
11 ".....	12	3	+
13 ".....	+	+	+
15 ".....	10	5	0
18 ".....	0	28	0
21 ".....	0	20	0
25 ".....	8	10	0
32 ".....	0	5	..
39 ".....	3	1	..
46 ".....	3	5	..
53 ".....	0	3	..
60 ".....	4	2	..
67 ".....	0	+	..
74 ".....	2	1	..
81 ".....	0	+	..
88 ".....	4	5	..
95 ".....	1	0	..
103 ".....	0	0	..

\*1 c.c.=0, 10 c.c.=+.

24 hours, the organism being detected in 1 c.c. up to the fifth day, and in 10 c.c. up to the 13th day. The bacterial results are shown in Table 23, and the *B. coli* results in Table 24.

*Experiment 218.*—Merrimac River water treated with ferrous sulphate. Range 0.020 to 20.1. The experiment was not complete when this was written.

TABLE 25.  
MERRIMAC RIVER WATER.  
(Bacteria per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000				
	Control	0.020	0.201	2.01	20.1
Start.....	600	.....	.....	.....	.....
1 day.....	1,300	425	700	300	250
2 days.....	600	500	44	85	130
3 ".....	4,900	2,400	900	1,500	1,700
4 ".....	.....	.....	.....	.....	.....
5 ".....	20,000	12,000	8,800	2,200	9,100
7 ".....	7,700	2,000	9,700	1,400	400
9 ".....	7,500	2,000	1,600	2,000	50
11 ".....	2,100	300	1,400	15,400	0
15 ".....	3,800	375	275	2,000	18
18 ".....	2,200	1,400	800	2,800	600
21 ".....	300	230	70	1,100	14
28 ".....	140	250	120	600	5
35 ".....	230	75	47	325	23
42 ".....	16	50	130	190	11
49 ".....	38	70	35	65	29
56 ".....	21	51	47	31	16
63 ".....	11	10	50	22	37
70 ".....	35	12	36	17	52
78 ".....	46	12	32	11	30
84 ".....	108	65	37	72	30
91 ".....	15	26	19	49	28

TABLE 26.  
MERRIMAC RIVER WATER.  
(*B. coli* per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000				
	Control	0.020	0.201	2.01	20.1
Start.....	20	..	..	..	..
1 day.....	26	15	6	2	3
2 days.....	8	23	9	6	3
3 ".....	19	9	15	20	4
4 ".....	7	4	6	11	4
5 ".....	3	4	0	2	3
7 ".....	0	7	0	0	8
9 ".....	3	0	2	14	0
11 ".....	4	0	5	..	0
15 ".....	11	16	6	6	7
18 ".....	0	1	2	10	0
21 ".....	0	2	0	0	0
28 ".....	0	0	0	0	0
35 ".....	15	6	3	0	0
42 ".....	12	0	4	11	3
49 ".....	3	3	1	2	5
56 ".....	7	9	0	1	1
63 ".....	1	4	0	0	0
70 ".....	10	5	16	16	10
78 ".....	8	11	4	6	4
84 ".....	0	0	0	1	0
91 ".....	0	15	14	17	3



The bacteria in all the bottles increased steadily for a few days, then decreased slowly. The maximum was reached in the control, 0.020, and 20.1 on the fifth day, in 0.201 on the ninth day and in 2.01 on the 11th day. *B. coli* were found in small numbers as late as the 78th day in the control, on the 91st day in the waters which had been treated. The bacterial results are shown in Table 25 and the *B. coli* results in Table 26.

*Experiment 158.*—Merrimac River water treated with sulphate of alumina. Range 0.134 to 0.537. Duration four days. The bacteria in all samples were reduced during the first 24 hours, and then began to increase about the third or fourth day. The results are shown in Table 27.

TABLE 27.  
MERRIMAC RIVER WATER.  
(Bacteria per c.c.)

ELAPSED TIME	ALUMINA—PARTS PER 100,000				
	Control	0.134	0.269	0.403	0.537
Start.....	950	.....	.....	.....	.....
6 hours.....	800	20	255	110	145
1 day.....	205	143	120	55	95
2 days.....	.....	248	176	155	70
3 ".....	1,200	400	790	10	2,800
4 ".....	.....	2,800	3,800	20,000	4,700

*Experiment 165.*—Merrimac River water treated with sulphate of alumina. Range 0.134 to 0.537. Duration 11 days. The bacteria in the control increased to a maximum on the third day and then declined. In all of the treated samples the bacteria were much reduced on the second day, but all showed a material increase from the third to the seventh days.

The *B. coli* results in this experiment are interesting, showing the occasional appearance of considerable numbers in all treated samples at intervals, with intermediate periods when the test organism was not detected. This phenomenon was probably due to errors in sampling caused by the precipitated aluminum hydrate, and would indicate that the *B. coli* were able to live in the precipitate and perhaps to increase. The bacterial results are shown in Table 28 and the *B. coli* results in Table 29.

TABLE 28.  
MERRIMAC RIVER WATER.  
(Bacteria per c.c.)

ELAPSED TIME	ALUMINA—PARTS PER 100,000				
	Control	0.134	0.269	0.403	0.537
Start.....	1,450	.....	.....	.....	.....
1 day.....	176,400	4,900	3,200	800	1,900
2 days.....	321,900	550	290	110	125
3 ".....	430,000	11,200	11,400	1,050	155
5 ".....	129,000	25,800	4,600	1,700	260
7 ".....	7,700	3,200	40,000	5,900	75,600
9 ".....	15,500	1,600	26,300	3,000	25,500
11 ".....	16,500	18,500	30,500	41,400	28,200

TABLE 29.  
MERRIMAC RIVER WATER.  
(B. coli per c.c.)

ELAPSED TIME	ALUMINA—PARTS PER 100,000				
	Control	0.134	0.269	0.403	0.537
Start.....	36	..	..	..	..
1 day.....	6	0	0	0	0
2 days.....	95	11	20	0	25
3 ".....	65	1	16	0	0
5 ".....	20	0	0	22	0
7 ".....	25	0	0	0	0
9 ".....	11	35	63	40	114
11 ".....	0	0	0	21	0

NOTE.—The occasional appearance of B. coli was probably caused by imperfect sampling due to the precipitated hydrate of alumina.

*Experiment 209.*—1,000 c.c. of Merrimac River water were placed in each of six jars, and thin sheets of copper, aluminum, lead, zinc, tin, and iron were inserted in each of six jars respectively, the seventh being retained as a control. Exposed metal surface 625 square c.c. Duration 132 days. The bacteria in the control showed a steady decrease after the first 24 hours. With the copper, the bacteria decreased during the first 24 hours, and then increased to a maximum on the eighth day, dropping

TABLE 30.  
MERRIMAC RIVER WATER.  
(Bacteria per c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Block Tin	Iron
Start.....	10,000	75,600	14,600	18,000	9,000	75,600	7,700
4 hours.....	11,000	700	11,000	12,700	4,100	15,200	18,000
8 ".....	10,200	180	15,500	9,300	6,200	24,000	14,700
24 ".....	9,700	430	8,500	2,900	5,200	14,000	5,500
2 days.....	2,500	75,600	7,000	4,500	11,000	6,700	7,300
3 ".....	800	850	5,000	24,000	14,500	4,300	1,400
4 ".....	800	.....	1,200	33,000	5,500	1,750	600
6 ".....	1,000	360,000	600	30,800	28,500	1,700	65
8 ".....	1,450	673,000	900	38,300	4,050	900	62
10 ".....	250	97,200	150	2,000	650	700	20
13 ".....	110	181,500	120	2,000	950	50	20
15 ".....	125	108,000	65	21,400	450	55	80
17 ".....	23	51,000	100	21,250	65	105	300
20 ".....	250	50,000	85	22,500	7	45	110
23 ".....	180	31,500	0	22,100	10	90	230
27 ".....	43	0	8	800	0	43	10
34 ".....	.....	310	55	1,100	0	12	360
41 ".....	180	10	10	1,200	.....	43	175
48 ".....	290	4	100	490	.....	720	430
55 ".....	225	2	35	400	.....	770	60
62 ".....	260	4	5	130	.....	60	60
69 ".....	200	0	0	55	.....	70	80
76 ".....	160	2	3	20	.....	550	42
83 ".....	350	10	0	10	.....	375	47
90 ".....	150	9	26	6	.....	120	110
97 ".....	125	4	12	14	.....	210	220
105 ".....	35	4	55	180	.....	350	130
111 ".....	75	30	65	500	.....	350	75
118 ".....	23	0	40	7	.....	75	36
125 ".....	38	13	60	10	.....	260	100
132 ".....	5	7	3	22	.....	52	85

TABLE 31.  
MERRIMAC RIVER WATER.  
(*B. coli* c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Block Tin	Iron
Start.....	32	52	18	10	5	4	25
4 hours.....	18	47	38	14	35	32	15
8 ".....	1	2	+	1	7	6	+
24 ".....	12	3	+	+	+	10	6
2 days.....	10	2	10	3	3	3	+
3 ".....	+	+	10	+	5	+	+
4 ".....	1	+	+	1	+	+	1
5 ".....	5	4	6	12	+	4	+
8 ".....	26	10	10	10	6	25	0
10 ".....	9	3	+	+	0	9	0
13 ".....	6	+	+	2	0	3	6
15 ".....	1	+	2	3	0	3	0
17 ".....	7	+	2	+	0	6	0
20 ".....	8	+	3	10	0	16	0
23 ".....	+	+	21	4	0	10	0
27 ".....	3	4	9	2	0	13	0
34 ".....	2	9	6	5	..	2	0
41 ".....	5	3	0	3	..	0	0
48 ".....	+	0	0	+	..	0	0
55 ".....	2	0	0	12	..	0	0
62 ".....	4	0	0	7	..	0	0
69 ".....	+	0	0	+	..	0	0
76 ".....	1	..	..	1	..	..	..
83 ".....	0	..	..	+	..	..	..
90 ".....	0	..	..	3	..	..	..
97 ".....	..	..	..	0	..	..	..
105 ".....	..	..	..	0	..	..	..

off slowly till the 23d day. With the aluminum there was a steady decrease in bacteria throughout the experiment. With the lead, the bacteria increased slightly to a maximum on the eighth day, remaining practically constant till the 23d day, after which they decreased steadily, with a slight secondary increase on the 105th day. With the zinc the bacteria increased slightly to a maximum on the sixth day and then declined rapidly, the water becoming sterile on the 27th day. The bacteria decreased steadily in the tin until the 41st day, when a small secondary increase began, lasting until the 125th day. With the iron, the bacteria decreased steadily, with small secondary increases from the 17th to the 48th days, and from the 90th to the 105th days.

The *B. coli* in all the samples decreased steadily, with the usual fluctuations, and disappeared from the zinc on the 10th day, from the iron on the 15th day, from the tin and aluminum on the 41st day, and from the copper on the 48th day, from the control on the 83d day, and from the lead on the 97th day. The bacterial analyses are given in Table 30 and the *B. coli* determinations in Table 31.

*Experiment 237.*—1,100 c.c. of Merrimac River water containing 1 per cent of sewage was placed in each of seven jars, and thin sheets of copper, aluminum, lead, zinc, tin, and iron were inserted in each of six of the jars respectively, the seventh being retained as a control. The exposed metal surface in each case was 625 square c.c. The experiment had only run 31 days at the time this was written. In the control, lead, and tin, the bacteria decreased steadily throughout the period of observation. In the copper a large increase occurred during the first four days, after which the bacteria declined steadily. In the aluminum the bacteria decreased steadily until the 15th day, when a secondary increase started lasting till the 23d day. With the zinc a considerable reduction occurred during the first 24 hours, after which the num-

bers increased till the third day, when a decline set in lasting throughout the period of observation. In the iron an increase occurred during the first 24 hours, after which a steady decrease was noted until the 15th day, when a secondary increase commenced which lasted until the 20th day.

The numbers of *B. coli* in the control increased largely during the first 24 hours, after which they decreased steadily until the water was practically sterile after 31 days. In all of the jars containing metals the *B. coli* decreased steadily, a slight secondary increase being noted on the 17th day in the aluminum. The fluctuations in the numbers of *B. coli* were quite noticeable, in a number of instances no organisms being found in one c.c. for a number of days, and then appearing in small numbers. The *B. coli* disappeared from the copper on the sixth day, from the zinc on the 10th day, from the tin on the 15th day, from the iron and lead on the 23d day, and probably from the aluminum on the 31st day. The bacterial analyses are given in Table 32 and the *B. coli* determinations in Table 33.

TABLE 32.

MERRIMAC RIVER WATER CONTAINING 1 PER CENT OF SEWAGE.

(Bacteria per c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Tin	Iron
Start.....	44,500	.....	.....	.....	.....	.....	.....
1 day.....	21,000	127,800	44,000	56,000	7,000	35,000	86,600
2 days.....	150,000	362,100	16,500	26,000	13,400	12,000	16,500
3 ".....	11,000	180,000	2,800	8,500	16,500	1,400	35,000
4 ".....	4,600	305,000	11,500	8,500	10,500	4,800	18,000
6 ".....	415	35,000	310	5,500	11,200	200	205
8 ".....	2,800	96,300	220	3,500	3,700	138	03
10 ".....	52	3,600	95	3,000	19	94	63
13 ".....	69	6,700	55	155	115	18	8
15 ".....	17	245	1,000	80	21	27	105
17 ".....	23	790	7,600	3	100	105	760
20 ".....	31	115	3,000	9	9	235	1,500
23 ".....	5	100	3,850	18	40	275	525
28 ".....	10	65	480	20	5	210	30
31 ".....	25	275	325	135	116	160	125

TABLE 33.

MERRIMAC RIVER WATER CONTAINING 1 PER CENT OF SEWAGE.

(B. coli per c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Tin	Iron
Start.....	3,500	3,500	3,500	3,500	3,500	3,500	3,500
1 day.....	38,000	2	1,370	140	22	3,100	650
2 days.....	250	.....	200	0	0	100	50
3 ".....	108	12	75	3	3	45	230
4 ".....	25	25	21	12	2	20	125
6 ".....	20	0	9	1	0	8	20
8 ".....	13	2	8	0	4	7	5
10 ".....	15	0	0	0	0	0	0
13 ".....	3	0	0	0	0	0	0
15 ".....	1	0	0	2	0	0	1
17 ".....	1	0	125	7	0	1	25
20 ".....	0	0	45	1	0	1	6
23 ".....	4	0	7	0	0	0	0
28 ".....	1	0	3	0	0	0	0
31 ".....	0	0	0	0	0	0	0



## RÉSUMÉ.

In the following résumé, the results of the copper sulphate experiments are brought together according to the amount of copper added to the water. This copper is expressed as the ratio of copper sulphate by weight to the weight of water, with the equivalent of metallic copper in parts per 100,000 in parentheses.

*Controls.*—In three experiments with polluted water, one showed a gradual decrease in bacterial contents, and the other two showed an increase, then a decrease which was followed by a secondary increase after some time. In four experiments with stagnant water, run for a short time only, one showed a decrease, one increased, one remained practically constant, and one increased and then decreased. In one experiment with driven well water, the bacteria increased, then decreased, and continued to fluctuate up and down during the 133 days the experiment continued.

In the one experiment made, *B. coli* under natural conditions showed a gradual decrease, but continued alive in 1 c.c. during 95 days. *B. coli* added to water as broth culture decreased, then increased. In three experiments using laboratory cultures of *B. typhosus*, the control was sterile after 24 hours in one case; in another a sharp decrease was noted till the third day, when an enormous increase commenced which lasted through 28 days; and in a third case the typhoid bacilli, which were present in large numbers, remained practically unchanged during 14 days.

*1: billion (0.0000253).*—In the one experiment made the bacteria followed the same laws as did the control.

*1:100 million (0.000253).*—The behavior of the normal water bacteria was observed in five experiments, four with polluted water and one with stagnant water. In four of the experiments the bacteria followed the same curve as in the control, and in the fifth they were nearly all destroyed at first, but the few remaining were able to multiply to large numbers.

In one experiment, *B. coli*, naturally present, acted much like the control, decreasing slowly but remaining alive some 88 days in 1 c.c. In one experiment with a laboratory culture a rapid decrease occurred, and the *B. coli* had disappeared at the end of six days.

Three experiments were made with laboratory cultures of *B. typhosus*. In one experiment large numbers survived three days, although the control died out in 24 hours; in another nearly all the *B. typhosus* were killed in 24 hours, but the very few remaining were able to increase to large numbers. In the third experiment the *B. typhosus* had become somewhat accustomed to life in water before the copper was added, and increased steadily during the 10 days they were under observation.

*1:10 million (0.00253).*—The normal bacteria were observed in eight experiments, four with polluted water, three with stagnant water, and one with deep well water. Three of the polluted waters acted like the control, but in the other nearly all the bacteria were destroyed at first, the few remaining, however, being able to increase rapidly. In the three stagnant waters the bacteria increased, although they failed to do so in one of the stagnant water controls.

Natural *B. coli* in one experiment decreased slowly, as they did in the control, but the numbers were usually larger than in the control. In one experiment with a laboratory culture the *B. coli* were killed in three days.

Two experiments were made with laboratory cultures of *B. typhosus*; in one the test organisms disappeared from 1 c.c. in three days, and in another nearly all were killed in four hours, but some remained alive in 1 c.c. till the 12th day, and tests of 10 c.c. showed them to be alive up to the 28th day. In one experiment with a culture of *B. typhosus* which had been grown four days in water, a steady increase occurred during the 10 days it was under observation.

*1: million (0.0253).*—The behavior of the water bacteria was observed in eight experiments, four with polluted water, three with stagnant water, and one with deep well water. In two of the polluted waters the bacteria followed the control, while in the other two they increased. In the three stagnant waters the bacteria increased, although they decreased in one of the controls. The bacteria in the well water followed the control.

Natural *B. coli* in one experiment decreased slowly, as did the control, but showed higher numbers than in the control. In one

experiment with a laboratory culture, the *B. coli* were killed in two days, testing 1 c.c.

In one experiment with a laboratory culture of *B. typhosus* the organism disappeared from 1 c.c. in 24 hours. In another experiment the *B. typhosus* were nearly all destroyed in six hours, a few were alive on the fifth day, but none were found in 100 c.c. on the seventh day. In the experiment where the typhoid bacilli were inured to the water, the numbers increased steadily during the 10 days they were under observation.

1:100,000 (0.253).—The bacteria were observed in seven experiments, four with polluted water, two with stagnant water, and one with deep well water. In three of the polluted waters nearly all of the bacteria were killed out at first, but the few remaining were able to multiply largely. In the other polluted water a large increase occurred at once. The numbers of bacteria in the two stagnant waters remained low for two days and then increased rapidly. In the well water the bacteria followed the same curve as in the control.

Natural *B. coli* followed the same curve as the control. With a laboratory culture of *B. coli* the organism disappeared from 1 c.c. in 24 hours. In one experiment with *B. typhosus* the organism disappeared from 1 c.c. in 24 hours. In another experiment all but a few were killed in six hours, but 10 c.c. tests showed some to be alive on the fifth day, and 100 c.c. tests showed some alive on the sixth day. In the experiment with water-grown typhoid, the organisms disappeared from 1 c.c. in 24 hours.

1:10,000 (2.53).—The total bacteria were observed in six experiments, four with polluted water and two with stagnant water. In two of the polluted waters the bacteria decreased gradually, and the waters became practically sterile after 89 and 131 days respectively. In one experiment nearly all of the bacteria were killed immediately, and in another the bacteria were practically all destroyed at first, but a few remained during 20 days. The bacteria in the two stagnant water experiments were practically all destroyed in 24 hours.

Natural *B. coli* acted like the control and remained alive in 1 c.c. after 103 days. With a laboratory culture of *B. coli* the organism disappeared from 1 c.c. in 24 hours. A laboratory culture of *B.*

typhosus and the special water culture of the same organism both disappeared from 1 c.c. in 24 hours.

1:1,000 (25.3).—The behavior of the normal bacteria was observed in three experiments with polluted water. Nearly all of the bacteria were destroyed in a short time in all of these experiments, but a few remained alive for 10 days, 20 days, and 68 days, respectively.

With a laboratory culture of *B. coli* and one of *B. typhosus* the organisms disappeared from 1 c.c. in 24 hours.

1:100 (253).—Two experiments were made with polluted water, in which all of the bacteria were killed in 24 hours.

The complete sterilization of water by allowing it to stand in a clean copper dish does not seem to be an accomplished fact; in only one of six experiments was the water completely sterilized, and that only after standing 55 days. The bacterial curves, taking both controls and copper dish cultures, seem to follow the same laws hitherto noted for standing water experiments. In two experiments the copper dish cultures consistently decreased in bacterial contents throughout, while in another experiment the numbers increased sharply and then declined slowly, both of which phenomena have been frequently observed in the various control cultures. In two experiments three entirely different waters were under comparative observation. The bacterial contents of each of these waters increased constantly, although one of them absorbed relatively large amounts of copper.

In four of the copper dish experiments, determinations of *B. coli* and of bacteria were made simultaneously. In these experiments the *B. coli* were present naturally, i. e., they came from fecal matter directly polluting the water, as opposed to experiments in which *B. coli* were added to the water in the form of a laboratory culture. Naturally we should expect to find the organisms under such conditions more resistant than in the case where we are dealing with cultures, and this proves to be the case. In one experiment the test organism disappeared from the water, testing 100 c.c. on the third day. In two other experiments the *B. coli* died out in the control dishes before they disappeared from the waters contained in copper, being found in 1 c.c. in one experiment on the 20th day, or five days after they had disappeared from 100 c.c. of the control; and in



the other experiment they were found in 100 c.c. on the 34th day, or six days after they had disappeared from a like volume of the control.

Three experiments were made with laboratory cultures of *B. coli* in sterile water standing in copper, and one experiment was made with *B. typhosus* under the same conditions. In two experiments *B. coli* were found in 1 c.c. on the sixth and eighth days, respectively, tests not being made in larger volumes in these experiments. In another experiment *B. coli* appeared in some numbers as late as the 10th day, and were found in 100 c.c. as late as the 40th day. In the experiment with a typhoid the bacilli were practically all destroyed after eight hours, and were not found in 100 c.c. after 24 hours.

From the two experiments made with sulphate of alumina and ferrous sulphate, these two salts appear to have about the same action on the bacteria and *B. coli* as have equal strengths of copper sulphate.

Judging from the results of experiments comparing metallic copper with other metals, all of the metals tested seem to be about equal in their effect on the numbers of bacteria in waters with which they are in contact. In one experiment the water in contact with zinc became sterile after about three weeks, while the numbers of bacteria increased in the water which was in contact with copper, and with all the metals, excepting zinc, a few bacteria were alive after 132 days.

Based on the disappearance of *B. coli* in 1 c.c. the metals in one experiment ranked:—zinc 10 days, iron 15 days, tin 41 days, aluminum 41 days, copper 43 days, lead 97 days; and in the other experiment:—zinc 10 days, copper 10 days, tin 23 days, iron 23 days, lead 23 days, aluminum 31 days.

#### CONCLUSIONS.

In conclusion, the writers believe that the treatment of water with copper sulphate or by storing it in copper vessels has little practical value, for the following reasons:

I. The use of any method of sterilization which is not absolutely effective is dangerous in the hands of the general user, tending to

induce a feeling of false security, and leading to the neglect of ordinary precautions which would otherwise be employed.

II. The removal of bacteria, *B. coli* and *B. typhosus*, by allowing a water to stand in copper vessels for short periods, while occasionally effective, is not sure, and the time necessary to accomplish complete sterilization is so long that the method would be of no practical value to the ordinary user. Furthermore, metallic copper seems to have little more germicidal power than iron, tin, zinc, or aluminum.

III. Although the removal of *B. coli* and *B. typhosus* is occasionally accomplished by dilute solutions of copper sulphate, these organisms may both live for many weeks in water containing copper sulphate in greater dilutions than 1:100,000; and in order to be safe dilutions of 1:1,000 must be used, in which case the water becomes repugnant to the user because of its strongly astringent taste.

IV. In some instances very dilute solutions of copper sulphate or colloidal copper absorbed from contact with clean metallic copper, appear to have a decidedly invigorating effect on bacterial activity, causing rapid multiplication, when the reverse would have been true had the water been allowed to stand the same length of time without any treatment.

## NOTES IN REGARD TO THE DETERMINATION OF COPPER IN WATER.

FRED B. FORBES AND GILBERT H. PRATT.

IN the course of a series of experiments carried on by the Massachusetts State Board of Health it became necessary to devise a method for separating and determining quantitatively small amounts of copper in water; such a method has been worked out and is published in detail in the "Standard Methods of Water Analysis" of the Laboratory Section of this Association.<sup>1</sup> It is the purpose of this short paper to give a brief outline of the method, together with some experimental results, and to call attention to certain statements that have appeared in print regarding the total disappearance of the copper in a few hours when applied to a water supply, and the impossibility of detecting it in the water by chemical tests.

The chemical test in general use for detecting copper in solution in small quantities is that mentioned by Moore and Kellerman in *Bulletin 64* of the Bureau of Plant Industry, and consists of adding potassium ferrocyanide to the solution to be tested, acidified by acetic acid. The sensitiveness of this test is shown by the following figures.

.00001 gm. copper, as copper sulphate, in 20 c.c. distilled water, equivalent to 1 part copper in 2,000,000, gave a very faint color, which it would be impossible to identify as the characteristic red of the reaction. 0.00002 gm. copper in 20 c.c. water, equivalent to 1 part copper in 1,000,000, gave distinctly the characteristic color. Both these strengths of copper solution were tested also in volumes of 100 c.c. in tubes, in order to observe the colors through a longer column than in the previous experiments, but with the same results.

The above tests were made with a solution of copper sulphate in distilled water, no other substances being present. It is well known that the presence of iron seriously interferes with the test, and it has been found that the coloring matter of natural waters is also a troublesome factor, so that in practice the sensitiveness of the test is much less than is indicated by the figures obtained with solutions in dis-

<sup>1</sup> *Jour. Infect. Dis.*, 1905, Supplm. No. 1, p. 1.

tilled water. A considerable quantity of water can be concentrated to small volume in order to increase the value of this test, but it then becomes necessary to provide for the removal of iron and organic coloring matter, or the results obtained are of doubtful significance.

Among various published accounts of the treatment of water supplies with copper sulphate, the writers have noticed in several cases the statement that after a few hours no copper could be detected in the water by the most delicate chemical tests, but have failed to see in any instance an account of the manner of making these tests, or any figures to prove their accuracy.

Owing to the unreliability of these qualitative tests for very small amounts of copper in natural waters, and to the necessity of obtaining quantitative results for purposes of comparison, it has been found that the only satisfactory way is to concentrate a large quantity of the water under examination, separate out the copper in proper condition for electrolysis, and deposit it as metal on a platinum electrode and weigh it.

The amount of water which is concentrated depends upon the amount of copper suspected to be present. Such an amount as will yield a weight of from 5 to 10 milligrams of metallic copper is preferable, as this amount of copper adheres well to the inside of the platinum dish which serves as an electrode, and can be weighed with accuracy on an analytical balance.

If the amount of copper in the water is very small, however, it is necessary to be content with a less weight, in order to avoid concentrating a very large volume of water.

It is the custom in the work of our board to collect three five-gallon carboys of water for each sample. Of this, 50 liters are concentrated for analysis, an equal amount being taken from each carboy and boiled down in three 10- or 14-inch porcelain dishes, with the addition to each dish of a little hydrochloric acid and 5 or 10 c.c. nitric acid. When concentrated as far as possible, the residues are united in one small dish, nitric acid being used to rub off the scum of organic matter which adheres to the sides of the dishes, and after further concentration the whole is treated with strong sulphuric acid and fumed over a lamp for some minutes. By this process, all



silica is rendered insoluble, much of the organic coloring matter is destroyed, and the metals present are converted to sulphates. After dilution and filtering, the excess of sulphuric acid is neutralized, and hydrogen sulphide added to the sulphuric acid solution. By this means the rest of the coloring matter is left behind, together with zinc, if present, and practically all of the iron. Lead, if present, will have been previously removed as sulphate. The sulphides are dissolved in nitric acid, converted to sulphates by strong sulphuric acid, and any iron remaining is removed by ammonia. The filtrate from the iron is acidified with sulphuric acid and, as a precaution, is allowed to stand for some time, in which case any lead which remained after the two previous filtrations will deposit as sulphate. The solution is then treated with 10 c.c. of concentrated sulphuric acid and 1 gram of urea and placed in a platinum dish and electrolyzed, the inside of the dish becoming the cathode, while a coil of platinum wire is placed in the solution for the anode. Any zinc which might be present remains in solution; in fact, we have made use of this method for separating quantitatively considerable amounts of zinc from small amounts of copper, or *vice versa*.

When the copper is all deposited, the dish is washed, dried and weighed; then the copper is dissolved off the dish in dilute nitric acid, and the dish dried and weighed again. The difference in weight gives the weight of copper. There is occasionally a very small amount of organic matter, due to the decomposition of the urea or organic matter in the solution adhering to the dish, and insoluble in nitric acid; hence the manner of weighing, instead of taking the weight of the empty dish before the electrolysis.

The solution of the copper in nitric acid may be made ammoniacal, when the characteristic blue color due to copper will appear. It has been found that 0.0001 gm. of copper in a volume of 20 c.c. gives a faint blue color by this test, while 0.0002 gm. copper gives a very distinct color. This represents a sensitiveness of 1 part in 200,000 and 1 part in 100,000, respectively. A set of standards may be made up with known amounts of copper in this condition and the copper obtained by electrolysis can be read on such standards, though 0.0001 gm. copper is the smallest amount that can be detected; and it is impossible to interpolate between successive 10ths of a milli-

gram. The test is of service, however, in verifying the result when the weight of copper obtained is only a few 10ths of a milligram.

Following are a few results obtained by the method with standard solutions, and also with natural waters to which known amounts of copper were added. In every case a considerable quantity of water was treated with the copper solution and then concentrated and carried through in the same manner as an unknown.

TABLE 1.  
EXPERIMENTS WITH SOLUTION OF COPPER SULPHATE IN DISTILLED WATER.

Weight of Copper Taken (Gram)	Weight of Copper Found (Gram)
0.0006	0.0005
0.0009	0.0007
0.0021	0.0019
0.0047	0.0050

TABLE 2.  
EXPERIMENTS WITH A NATURAL SURFACE WATER TO WHICH KNOWN AMOUNTS OF COPPER SULPHATE  
WERE ADDED.

Weight of Copper Taken (Gram)	Weight of Copper Found (Gram)	Quantity of Water Concentrated (Liters)
0.0006	0.0008	50
0.0014	0.0017	50
0.0047	0.0048	34
0.0091	0.0085	34
0.0222	0.0202	16
0.0404	0.0360	16

These results show the method to be capable of separating and determining small amounts of copper with considerable accuracy. In studying the figures it appears that the percentage error is quite large, especially in the case of the smaller quantities. It must be borne in mind, however, that the absolute error is very small; in fact, in some instances as small as the analytical balance is capable of detecting. In the case of the larger quantities, an additional error is introduced by dividing the solution before electrolysis, in order to avoid having too heavy a deposit on the dish. All of the results were obtained without previous knowledge by the operator of the amounts of copper present, and the determinations were carried out in all respects as with unknowns.

TABLE 3.

This table shows a series of results obtained from samples of water from a large natural reservoir infested with algae, to which copper sulphate had been applied. In every case 50 liters of water were taken for analysis; the figures show the weight of copper found in each sample and its approximate dilution.

	Gram	Equivalent to 1 part Cu in	
Before dosing . . . . .	0.0001		500,000,000
1 day after dosing . . . . .	0.0025	" " I " " "	20,000,000
2 days " " . . . . .	0.0016	" " I " " "	31,250,000
3 " " " . . . . .	0.0012	" " I " " "	41,666,000
7 " " " . . . . .	0.0006	" " I " " "	83,333,000
13 " " " . . . . .	0.0004	" " I " " "	125,000,000
27 " " " . . . . .	0.0002	" " I " " "	250,000,000

These figures show clearly the necessity of operating on considerable quantities of the sample if reliable quantitative results are to be obtained. They show also the fallacy of depending on the ordinary qualitative tests to prove a water free from copper. The most delicate of the various tests would fail to reveal the presence of the metal in the original concentration in any of the foregoing samples, while if the water is subjected to concentration, some provision must be made for the removal of iron and organic coloring matter, or the accuracy of the test is vitiated.

It is believed that the method as devised is the most accurate and satisfactory one at the present time, and, though somewhat tedious, justified by the importance of thoroughly testing this comparatively new process of water purification before passing judgment on its value.

The method may also be used upon sand, the copper being extracted therefrom by means of nitric acid and the subsequent procedure being the same as with a water residue.

## A NOTABLE SOURCE OF ERROR IN TESTING GASEOUS DISINFECTANTS.

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THE testing of gaseous disinfectants for public health purposes is done for two general objects, in two different ways. The first object is that sought when the investigator determines from a large number of experimental tests the required amounts of gas and best methods of work, and then prescribes these for use in practice. The second object is to secure a check system by which each particular disinfection performed in practice is tested as a routine procedure by the exposure in the room of one or more test organisms, the death of which is required before the disinfection is officially approved. The two systems supplement each other, but the second is scarcely required if the disinfection is performed strictly according to a proper prescription, and is intended primarily as a test of the disinfectant rather than of the disinfection. For whatever purpose the tests are made, it is rather obvious that they should be so designed that the survival of the test organism should be good evidence that the room treated would have remained infected, had it been originally infected naturally by a patient suffering from an infectious disease, and also—*vice versa*—that the death of the test organism should prove that the room treated would no longer be infective, if it were infective before disinfection was done. It is of the greatest moment, therefore, that the condition of the test organisms, as well as their character, should parallel closely, or, if possible, be identical with, the condition of the infective agents which are, or are supposed to be, present in rooms occupied by infected patients.

The writer has had occasion to point out in previous articles<sup>1</sup> the probable position and condition of infective agents in naturally infected rooms. It is now his object to point out, from accumulated evidence, the requisites which should be demanded of test organisms

<sup>1</sup>*Am. Pub. Health Assoc. Rep.*, 1902, 28, pp. 209, 509; *Bulletin*, Vermont State Board of Health; presented at the Vermont summer school for health officers, 1903.



used in testing disinfectants, these requisites being based on the premise above given, i.e., that the test organism should parallel in condition, as well as character, the infective agents which it is desired to destroy.

It has been pointed out by a number of investigators that the efficiency of gaseous disinfectants, chlorine, sulphur, formaldehyde, carbolic acid vapor, etc., is dependent very largely on the humidity of the atmosphere in which they act. In saturated atmospheres remarkably small amounts of these gases are efficient. In dry atmospheres, they are practically inert, even if present in relatively large quantities. But a most important point is this, that a dry gas, acting upon a moist organism, kills it just as surely as does a moist gas acting upon a dry organism. This very simple fact has led to much of the confusion of results in work carried on by different observers, who, attempting to obtain similar results by similar methods, obtained contradictory results because of the differences in the degree of moistness of the test organisms respectively used; while another set of contradictions has resulted from differences in the humidities of the atmosphere in different tests. With two such important variables almost entirely overlooked in most "practical" disinfection tests of gaseous disinfectants, it is not astonishing that one observer records excellent results from the use of a method which, in the hands of another, utterly fails.

The work upon which this paper is based was done in the attempt to reconcile two sets of absolutely contradictory results obtained, one set in Boston, the other in the hands of a high authority not far away from Boston. A third, and more unusual, source of error was incidentally discovered—the drying of test organisms to an unusual extent; the discovery resulting finally in a statement from one observer that the sum total of favorable results he had so far obtained was absolutely worthless, since his controls themselves did not survive the period of drying employed, without the use of any disinfectant at all.

Briefly summed up, the facts are that test organisms, fresh and moist, are very susceptible to small quantities of disinfectant gas, dry or moist; the same organisms, fresh but dried, are extremely resistant to the same amounts of dry gases; while, finally, the same organisms, dried

for a week or more, lose vitality to such an extent that even small amounts of gas, not necessarily at high humidities, will kill them. The writer has exposed in the same room, under identical conditions and at the same time, the same organisms on filter paper, and kept all night, one-half in such a manner as to dry thoroughly, the other half in such a manner as to remain moist. The moist organisms were all killed, the dry organisms all survived. Test objects prepared so that an intermediate stage of dryness was reached, behaved irregularly, some surviving, some perishing. Moreover, organisms (*B. pyocyaneus*) prepared on successive days, in such a manner that at the end of a week, all could be exposed under identical conditions to the same gas, gave results which showed that those prepared immediately before the exposure (i. e., still moist) were killed, those prepared two, three, and four days before (i. e., well dried, but still fresh) survived; while those prepared five, six, and seven days before were killed in numbers proportionate roughly to the age of the specimen, those longest dried showing the largest proportion of killed.

The writer's object in submitting this statement at this time is to point out certain sources of error which have in the past given to those concerned in selecting methods of disinfection a bewildering set of contradictory data to digest. Some of those to whom such contradictory results have been submitted have naturally enough become disgusted with all results of the same nature. Nor is it to be wondered at, when, for instance, one city adopts, on bacteriological evidence, five ounces of formaldehyde per 1,000 cubic feet, and another city, also on bacteriological evidence, has announced officially that over 70 ounces per 1,000 cubic feet is not uniformly efficient. The question will naturally arise: What character and conditions should a test organism have to yield a satisfactory and conclusive result? The answer is involved in what has been said already. Categorically, it should be fresh—not more than two days dried; and it should be really dry; while the species used should be those encountered in actual practice, or non-pathogenic forms, carefully tested and selected to show parallel resistance to those which it is desired to kill in practice. The reason for selecting dry organisms, dried, however, not more than two days, is simply that such organisms represent the conditions which those infective agents naturally distributed in the

infected room will most probably present. Any organisms in the room freshly deposited by the patient just before death or removal, are likely to be moist, hence are likely to be killed readily. Those deposited by the patient a week or more before are likely to be dead, or at least dried to a point of low resistance, and are also readily killed. It is therefore the organisms that were thrown out by the patient from one to seven days before his death or removal that will usually prove the more resistant, and it is to these that the disinfectant must pay attention. It is obvious that organisms of like age and dryness should be used for the tests. To kill organisms in this condition high humidity of the atmosphere is required if efficiency, with economy of gas, is desired.

In practice, bacteria dry more quickly on glass than on cotton or filter paper. This is probably the chief reason, if not the only one, why it has been noted by various observers that organisms dried on glass are more resistant (if not dried too long) than those dried on the other materials under like conditions. Hence glass objects for test organisms have, in the writer's hands, given the most generally reliable and uniform results.

## METHODS OF BACTERIOLOGICAL EXAMINATION OF MILK.

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THE bacteriological examination of milk is rapidly becoming one of the essentials in the maintenance of public health. For years milk supplies have been inspected and tested to chemical standards alone, with the result that watered, adulterated, and preserved milks have been practically driven from the markets. These chemical tests are necessary, and only fail in that they do not go far enough, since a milk impure and unwholesome on account of bacterial growth, or a milk from a diseased animal, cannot be detected by them.

The bacterial count and the microscopical examination of the milk sediment reveal whether the milk has been properly handled or not, and, to a great extent, the condition of the animal from which it came.

It so happened that a conviction of the importance of this examination came to several cities at about the same time, and as no methods of milk examination had been agreed upon among bacteriologists, each laboratory started the work independently as best it might. As a result, a large number of laboratories make routine bacteriological examinations of milk, but cannot compare results, because of differences in methods.

The subject is of so much importance that these various methods of technique and differences of apparatus ought to be compared, in order that the best may be chosen; and definite, uniform methods for routine examination should be agreed upon.

It is with this object in view that the following technique and apparatus, developed at the Boston Board of Health Laboratory, under the direction of Dr. H. W. Hill, are offered for comparison and criticism.

Before milk work was begun, Dr. E. H. Wilson, of Brooklyn, courteously permitted Dr. Hill to examine and collate the replies from about 15 prominent laboratories doing milk work, made



in response to a circular letter containing questions concerning technique, which Dr. Wilson had sent out. Although the replies showed wide differences in technique, even among workers devoted largely or solely to milk examinations, the technique in Boston was based on the consensus of the opinions given and modified from time to time as the work developed and became familiar to us.

For collecting samples, we have designed an apparatus using test tubes as containers; the samples, after thorough mixing, are transferred to the test tubes by means of large glass pipettes, a clean sterile pipette being used for each sample. The case for carrying the samples is made of copper, with double walls interlaid with half-inch felting, divided into three compartments, the central one for samples, the other two for ice. The test tubes are arranged in small racks, made of copper tubing weighted with a strip of lead and padded with rubber. Each rack holds four tubes. Holes in the bottoms of the partitions allow ice-water to circulate freely around the lower ends of the tubes. When this outfit is iced and closed, a constant temperature of 34° F. is maintained. The pipettes are carried beneath the sample case in a detachable copper box, adapted for sterilizing and divided into two compartments, the upper for clean sterile pipettes, the lower for the pipettes after use.

The use of the test tube has been adopted, in preference to the use of bottles, for the following reasons, dependent in most cases on the long slender shape of the tube:

1. Economy of floor area in the collecting case.
2. Avoidance of the necessity for two or more layers of containers, the lower layer of which would be always difficult of access.
3. The facility for maintaining low temperature by the circulation of ice-water about the lower ends of the tubes.
4. The ease with which all the usual washing, sterilizing, and general handling of test tubes can be done, since the test tube is a regular piece of ordinary apparatus, involving no departure from the ordinary routine in all the usual manipulations.

In plating, we have followed the methods recommended by the Bacteriological Committee.<sup>1</sup> We have found a dilution of 1:10,000 best suited for routine work. When examining samples recently

<sup>1</sup> *Jour. Infect. Dis.*, Suppl. No. 1, 1905, p. 1.

taken from individual cows or from a milk supply known to be fairly pure, we dilute 100 times. When the microscopical examination, which will be described in detail later, shows a milk crowded with bacteria, we dilute 1:1,000,000. The lowest count we have recorded is 300 bacteria to 1 c.c.; the highest 640,000,000.

For dilution water, square eight-ounce bottles have been found more easy to handle and more economical of space than other forms of bottles or flasks. Straight sided 1 c.c. pipettes are more easily handled than those with bulbs. They can easily be made from small glass tubing and calibrated in the laboratory.

For a medium, we use agar-agar made according to the directions of the Bacteriological Committee. A few changes have been made after careful comparison of results with varying acidities and percentages of agar. We have the best results with a 1 per cent agar, reaction +1.5.

The additions of lactose or litmus to the medium has not, so far as we have tried it, proved of any special advantage.

We do not use gelatin, on account of the difficulty of maintaining uniform room temperature, and the length of time which must elapse before a report can be made.

The agar plates are incubated in a saturated atmosphere for 24 hours at 37° C.

Comparison of plates grown at room temperature with those grown at 37° C. has at times shown great differences in the number of colonies developing in duplicate plates. These differences, however, have not been uniform, the higher or lower temperature developing more colonies according to the nature of the organisms present. Incubation at 37° C. has been adopted, since it allows a much quicker report; it gives each sample the same treatment at a regulated temperature; and it allows a fair comparison of results obtained over long periods. If the plates are incubated 48 hours, a slightly higher average count will be obtained, not enough, however, materially to change the report. In many plates the count is lower at the end of 48 hours on account of small colonies becoming obscured in the growth of larger ones. There are also more spreaders, which means a greater loss of counts.

Porous Petri dish covers, suggested by Dr. Hill and since recom-

mended by the Bacteriological Committee, have proved very efficient in reducing the number of spreaders. By their use in our work, spreaders have been reduced from 35 per cent to practically none.

Spreading under glass covers seems to be caused principally by water condensing on the inner surface of the cover and on the surface of the agar. The first fault can be partially overcome by inverting the dish, but the second seems to be unavoidable except by using the porous covers. With the glass covers, the bacteria in the surface colonies multiply rapidly in the film of condensation water, often spreading over the whole surface, and thus making a count impossible. The dry porous earthenware covers absorb this condensation water, still leaving the atmosphere saturated, as is proved by the quick growth and large size of the colonies. It is not necessary to invert the dishes; the covers are cheaper and more durable than glass, and they can be marked with lead-pencil, the marks erasing easily. Organisms having an inherent tendency to spread from unusual motility are not prevented from spreading by the use of these covers.

With the porous covers, as with the glass, each additional day's incubation shows an increase in the number of spreaders, due partly at least to the fact that the moisture gradually fills the interstices so that they do not absorb as readily as at first; but, day by day, the spreaders are much smaller in number than with glass covers.

It is quite essential to the best results that the porous covers should be washed as seldom as possible. In sterilizing them, the process should be prolonged over the time necessary to kill the organisms, in order that the covers may be thoroughly dry.

Our counting apparatus is simple and inexpensive. A circle, four and one-half inches in diameter, divided into 10 equal segments, is cut into the surface of a child's school slate; the lines are then filled with red lead, against which any colonies lying immediately over them, are easily seen. The surface of the slate, which tends to become gray, with time and use, may be kept black by occasionally rubbing with a little vaseline. The Petri dish is placed, uncovered, bottom down over the circle. A wooden box, six by six by five inches, with open bottom, glass front, and a four-inch circular opening in the top, the wooden parts painted black within and without to avoid refraction of the light, is placed over the plate and centered.

A common four-inch reading glass, magnifying about two diameters, fits over the opening in the top of the box, thus protecting the plate, keeping a constant focus and leaving both hands of the operator free. A tally record for adding and recording the counts by a simple pressure of the thumb for each colony seen, completes the outfit.

The advantages of this counting apparatus may be summarized as follows:

1. Cheapness.
2. The lens is held at the proper focus, leaving both hands of the operator free.
3. The whole field is exposed to view, so that there is no danger of counting the same colony twice.
4. The radial division into 10ths makes it easy in a crowded plate to obtain an approximate estimate by multiplication of the count of one or two representative sections.
5. The plate is so well protected from contamination that it may be counted face up with the cover removed.

For the microscopic examination of milk we first obtain the sediment from a known quantity by centrifugalizing; Stewart, of Philadelphia, uses an apparatus by means of which a large number of samples may be treated at the same time.

A modification of this consists of an aluminum disc and cover, the whole being 10 inches in diameter and  $\frac{5}{8}$  of an inch in depth.

This disc is fitted to hold 20 small tubes arranged radially. The tubes hold about 2 c.c. each. Both ends are closed with rubber stoppers. By the use of these tubes, the whole sediment from a known quantity of milk is obtained, and may be spread over a given space. We have arbitrarily adopted a space of 4 sq. cm.

For smearing the sediment, slides nine by two inches of common window glass are convenient. One of these may be ruled with blue pencil into 11 spaces, each 2 by 2 cm., leaving a space 2 by  $1\frac{1}{2}$  cm. at the top of each for the sample number and a similar space at the lower end for comments on the microscopical examination. By stopping the tubes at one end before opening the sample case, the time of exposure of the samples to room temperature is lessened. Each sample need be exposed only a few seconds while filling the tube, and immediately replaced in the case.



The milk sample is shaken 25 times, the tube is filled from the test tube by pouring, then stoppered, and finally inserted into its properly numbered receptacle in the disc. We centrifugalize 10 minutes at a speed of 2,000 to 3,000 revolutions a minute.

To obtain the sediment with least disturbance, first remove the stopper at the inner or cream end, then, holding the tube with the cream end downward, remove the cream with a platinum loop and pour the milk out; lastly, still holding the cream end down, carefully remove the other stopper with the adhering sediment and smear the sediment evenly, with a drop of sterile water, over the measured space on the glass slide, rubbing the stopper directly on the glass until all the sediment is removed. Dry with gentle heat and stain with methylene blue.

The examination of a properly prepared milk sediment under the microscope with a  $\frac{1}{12}$  oil immersion lens gives a very good idea as to the number of bacteria present. We began this examination with the intention of looking for pus and streptococci alone. The variation in the number of bacteria in different samples was, however, so apparent under the microscope that it suggested this form of examination as a more convenient and quicker method than plating for determining the bacterial content of milk, or at least for eliminating samples comparatively free of bacteria.

We determined to test the question thoroughly by comparing the microscopical estimate with the actual count as obtained from the plates. The comparison was carried out very carefully with over 2,200 samples, each sample being subjected to the double test, i. e., plating and centrifugalizing; the microscopic estimate was made before the plate was counted and an error of less than 1 per cent was made in passing (as below 500,000 bacteria to 1 c.c.) milks which in the plates showed above this limit. Over a third of the total error occurred in the first 420 samples, before the method was fully developed.

This method would be useless in examining very clean milks for certification, as the lowest limit of accuracy would probably be around the 100,000 to 1 c.c. mark. Perhaps, by obtaining the sediment from a larger amount of milk, the test could be made more delicate.

If the microscopic estimate is made before the samples are plated,

it is hardly necessary to plate those samples which are manifestly within the law's requirements. Besides such cases, many milks are obviously above the legal limit; these are plated in order to confirm the microscopic examination, and to have definite figures for legal purposes. Besides those entirely below and above, there are still others about which the examiner is in doubt. These of course need to be plated. Finally, of those apparently above the law, there are occasional samples which on plating run below it. It is still a question in such cases whether the plate or the microscope is the more correct. This group, although a very small one, makes the plating of high count milk more necessary, since it would be inadvisable for legal purposes to go into court on the microscopic estimate alone.

It is easy to determine, through the microscopic examination, what dilution will be necessary in plating, in order to ascertain correctly the number of bacteria present.

When examining the sediment for pus, since pus cells and dead leucocytes are identical, it is impossible to differentiate them under the microscope by the appearance of the individual cells. While it is not uncommon to find milks with these cells nearly absent, most milks have at least three or four such cells to the  $\frac{1}{12}$  oil immersion field.

Opinions as to the number of such cells required to indicate pus differ. Professor Bergey, of the University of Pennsylvania,<sup>1</sup> says: "There is still no agreement among bacteriologists as to the number of cells in a specimen that will justify the diagnosis of the presence of pus. The number of cells in a field of the  $\frac{1}{12}$  immersion lens is taken arbitrarily at 10. This number of cells per field may not always indicate pus, but it is believed that in the majority of instances it does indicate the presence of pus in milk derived from individual cows." Stewart, of Philadelphia, with the sediment from 1 c.c. of milk spread over a surface of 1 sq. cm., allows 23 cells to the  $\frac{1}{12}$  immersion field before reporting pus. On account of the large amount of this milk with high cellular content at present in the markets, and the not wholly unreasonable doubt as to whether such milk is injurious to the public health, it has seemed fairest to all concerned, while fully recognizing that some abnormal condition

<sup>1</sup> *Bulletin 125, Comwltth. of Penn., Dept. Agric., 1904.*

must be present in the animal yielding such milk, to adopt a standard for market milk, for the present, allowing not over 50 cells to the  $\frac{1}{12}$  immersion field (spreading the sediment from 2 c.c. of milk over 4 sq. cm.). So little of the sediment is seen at once with the high power that it is well to confirm the diagnosis of pus by making a thorough examination of the whole surface with a low-power lens, to determine how uniform a smear has been made. By using an eye-piece micrometer, ruled in squares, the relation of the area of one square to that of the  $\frac{1}{12}$  immersion lens being previously calculated, a count may be made with the low-power lens.

Streptococci are, in our experience, seldom found to any great extent by direct microscopical examination. Occasionally a sample will be found crowded with long chains. More often streptococci, if present, are in the form of diplococci or very short chains. In any examination where streptococci, diplococci, or cocci are found in the sediment, and the plate from the same sample contains colonies resembling streptococci colonies in excess of a count of 100,000 to 1 c.c., we transplant these colonies to broth to see if chains will develop. We first make and record an estimate of the number of such colonies present, then transfer from 10 to 50 of them to broth, and grow for 24 hours at 37° C. Streptococci in small numbers are present in most market milk.

We condemn a milk for streptococci when these three tests are all positive:

1. Microscopic examination of the sediment showing streptococci, diplococci, or cocci.
2. The plate from the same sample showing colonies resembling streptococci colonies, in excess of 100,000 to 1 c.c.
3. The broth culture from these colonies showing streptococci alone or in great excess of the other bacteria present.

In conclusion, a word on the correlation of temperature and count. At first thought, one would always expect to find a high bacterial count in samples showing a high temperature, and a low count in low temperature samples, and if all the milk in question were of the same age and had been kept continuously at the temperature found, this would generally be the case. But it is easy to see that a high temperature might accompany a low count on milk only a few hours

old which had not been properly cooled, while a low temperature and a high count are frequently found together in milk recently cooled which previously for 12 to 36 hours had been kept warm, as is often the case during transportation as practiced in some parts of the country. Thus, by taking temperature alone it is impossible to state whether or not a milk is good from the bacterial standpoint, and if milk is judged by the temperature standard alone, much that is good may be condemned, and very much that is poor will be kept on sale. Taking temperatures is important, and tells us how the milk is being cared for at time of sampling. By making bacterial counts, we find how the milk has been cared for up to the present, any lack of cleanliness or care being recorded by the corresponding increase in bacteria. By centrifugalizing and examining the sediment, we can estimate the bacteria present, and detect milk which on account of disease or dirt is unfit for use as food.

All of these methods of examination have been carefully worked out with checks and controls. Realizing that there is still great room for improvement in many ways, we recommend them as a system which has been reasonably successful in practice, for city laboratory work where quick reports are needed as well as the accurate examination of large numbers of samples. We do not offer them as developed to a final point of simplicity and efficiency; our hope is only that their presentation here may serve to provoke discussion and to secure ultimate agreement on uniform and efficient methods.



SUGGESTIONS FOR CHANGES IN THE SCHEDULES  
FOR MAKING BROTH, GELATIN, AND AGAR,  
RECOMMENDED IN THE LAST REPORT OF THE  
COMMITTEE ON STANDARD METHODS OF  
WATER ANALYSIS.<sup>1</sup>

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*Suggestion 1.*—In preparing broth, gelatin, and agar, transfer the direction to “adjust to the required reaction,” from its present position to precede immediately the direction for heating over the water bath for half an hour.

The reason for this change is that, in order to secure uniformity in successive lots of media, it is well that *all* heating should be done at a uniform reaction. If the recommendations as they stand now are followed, the half-hour's heating on the water bath is given before the adjustment to a fixed reaction, i. e., at the casual reaction which the meat infusion may happen to have, further modified somewhat by the addition of peptone in all cases, and, in the case of gelatin, very much modified by the presence of gelatin. If, however, the change here suggested be adopted, the reaction at which all heating is done will be the same throughout for all media *which are to have the same final reaction*. It will be remembered that the earlier recommendations of this Committee called for neutralization before heating, and this provided that the reaction at which all heating was done should be the same throughout for all media, *without regard to the final reaction*. My suggestion, if adopted, will return as closely as may be to uniformity under the modified conditions as regards the final reaction now generally adopted, i. e., the adjustment to a final reaction without previous neutralization.

*Suggestion 2.*—In preparing broth, gelatin, and agar, make the period of boiling over the free flame five minutes instead of two

<sup>1</sup> *Jour. Infect. Dis.*, 1905, Suppl. No. 1, p. 1.

minutes as in the present recommendation. This also is a return to the recommendations formerly made.

The reason for this change is, that it seems to be a fairly common experience that two minutes' boiling is hardly sufficient to precipitate thoroughly the albumens present at the reaction at which the boiling is done. Five minutes' boiling, on the other hand, is usually sufficient for precipitation, and obviates subsequent precipitation during sterilization.

*Suggestion 3.*—Since, in the adjustment of agar media by titration, some little difficulty is encountered at times, if it be done after the addition of the 3 per cent agar, and especially if the titration process be prolonged, because of the agar becoming cold, it is at times convenient to adjust the reaction of the meat infusion plus peptone before adding the agar. If this be done, the reaction to which the meat infusion is adjusted should be double that which is desired as the final reaction. The subsequent addition of the agar in 3 per cent strength, as recommended, brings the reaction, as well as the percentage of peptone and of meat infusion constituents, to the proper point. Since it is difficult to see that the adjustment of the agar reaction before or after the addition of agar can make any difference in the composition of the medium, it would seem that an alternative method might here be provided without infringing on the principle of securing strict uniformity. The 3 per cent agar being neutral, exactly the same amount of alkali is necessary to secure the same final reaction from either the meat infusion plus peptone, (double strength) brought to double the final reaction, and then diluted one-half by the 3 per cent agar or from the whole agar medium (final strength) brought directly to the final reaction.

Summed up these suggestions would be as follows:

1. In the present form of recommendations for gelatin and agar, p. 108 of Supplement No. 1 of the *Journal of Infectious Diseases*, strike out steps 11 and 12; and insert them again, renumbered 9 and 10, respectively; strike out 9 and 10 as they now are, and reinsert them, renumbering them 11 and 12, respectively.

2. In step 13, strike out "two minutes" and substitute "five minutes."

3. Insert a footnote, referring to steps 8, 9, 10, as renumbered in accordance with the above suggestions, to read:

If preferred, meat infusion and peptone of double strength, intended for the preparation of agar media, may be adjusted to twice the final reaction desired, before the addition of the 3 per cent agar. The addition of the agar should then be made between steps 10 and 11 (as renumbered).

## A DEVICE FOR FILTERING TOXINS, ETC., BY THE USE OF WATER PRESSURE.

HIBBERT WINSLOW HILL.

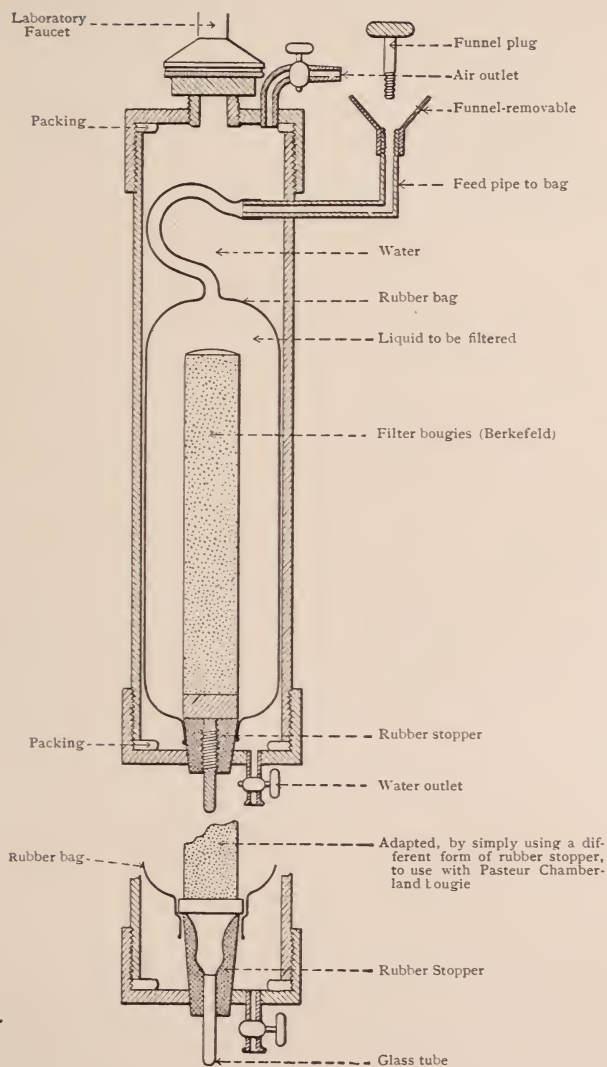
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THE use of gas or air pressure to drive toxins, or other bacterial fluids, which it is desired to filter, and at the same time to sterilize through bougies of porcelain etc., has been common for a long time. The ordinary aspiration filter is, of course, really a pressure filter, the limit of pressure being rather less than one atmosphere, and depending on the degree of exhaustion obtainable. But direct air or gas pressure—secured say by compression—has always presented, besides the mechanical difficulties, chemical or physical difficulties, dependent on the pressure-modifications of the action of the gases used upon the liquids to be filtered.

It occurred to the writer that the interposition of a thin sheet of rubber between the surface of the liquid to be filtered and the liquid (air, gas, or water) which was used as the vehicle to transmit the pressure would at once remove almost all the objections which could be brought against pressure filtration. Because of its relative density and inelasticity, water presents so many mechanical advantages over air, that attention was concentrated wholly upon the use of water pressure.

The device illustrated by the accompanying drawing is extremely simple in principle. A strong metal cylinder of appropriate size to contain the bougie, and a rubber bag, made to surround the bougie and to contain, say from one-half to one liter of the liquid to be filtered, are the sole requisites besides sufficient water pressure. Water may often be obtained from the laboratory tap under pressure of from 45 to 90 lbs.—say from 3 to 6 atmospheres. A small force pump may readily be provided which will supply 200 to 300 lbs. pressure. This water is admitted to the cylinder upon the outside of the rubber bag containing the liquid to be filtered. The pressure drives the rubber inward upon the contained liquid, and so upon the contained bougie, with a perfectly adjusted and uniformly dis-





tributed pressure. There is nothing to tear the rubber, which may be very thin; every drop of the contained liquid is filtered; the filtration is very rapid; and there is no question introduced as to the effect of the exposure of the liquid to air or gas under pressure, for it has had no such exposure. To reduce the pressure from say 200 lbs. to normal, it is only necessary to shut off the source of the water pressure by turning a tap, and to allow the escape of a few cubic centimeters of water from the cylinder. The rubber bag may be refilled after each emptying very readily.

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## ON THE USE OF METHYLENE BLUE IN TESTING SEWAGE EFFLUENTS.

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THE chief aim of sewage purification processes is to change the organic compounds of sewage into such a stable form that they will no longer tend to undergo offensive putrefactive changes. In special cases, as, for example, when effluents are discharged into tidal waters where shellfish are exposed to pollution, a high percentage removal of bacteria may also be necessary. In most instances, however, the purification plant will have done its work if it produces an effluent free from decomposable organic matter. To what extent this end is accomplished is one of the paramount questions in the control of any plant.

It is more and more clearly recognized that the intelligent operation of sewage works demands systematic analyses of the sewage and effluent at each of the principal stages in the process. The examinations which are made at different plants, however, vary widely in their precision. At the larger works complete analyses are carried out, including total organic nitrogen, suspended and dissolved, free ammonia, oxygen consumed, suspended and dissolved, nitrites, nitrates, dissolved oxygen and total solids, suspended and dissolved, with the loss on ignition. In other cases the effluents are merely examined by a rough test for putrescibility.

There is likely to be an increasing demand for more and more exact scientific control of sewage filters as sanitary standards become increasingly rigorous; rule-of-thumb methods, here as elsewhere, have probably had their best day. No conception can be formed of what goes on in a septic tank or a trickling filter or a contact bed, without knowledge of the amount and nature of the suspended matter. For example, an effluent in itself stable might easily produce in a stream a deposit of solid sludge which would eventually cause a serious nuisance. It is very helpful to the operator of a plant to know the general condition of the nitrogen and the available oxygen content at each stage of the purification process. All the analytical data noted above should therefore be obtained by the analysis of composite samples whenever possible.

The ordinary methods of analysis are, however, insufficient by themselves to furnish all the information necessary with regard to the character of a sewage effluent. Our knowledge of sewage chemistry has not yet made it possible to discriminate clearly between putrescible organic matter and those more stable "humus-like" compounds which do not undergo putrefactive decomposition. With intermittent filters, effluents are obtained which contain only a small amount of organic matter of any sort; and the purity of such effluents is easily demonstrable by oxygen consumed and nitrogen determinations. With effluents from contact and trickling filters, on the other hand, the ordinary analytical data do not offer a complete criterion of quality. An effluent may contain a considerable amount of organic nitrogen and may give a high value for oxygen consumed and yet prove of excellent quality because its organic matter is in a stable form. In such cases it becomes necessary to supplement sanitary analysis by some practical test of keeping quality. Such a test, if its technique is easy, can be made to advantage at more frequent intervals than the more elaborate chemical examinations; and in small plants where the latter are out of the question it alone may prove of considerable value.

Although the terms putrescible and non-putrescible stand for quite definite characteristics, yet the separation of effluents of various degrees of purity into these two classes depends to a considerable extent upon the test employed for the purpose. Crude sewage is



undoubtedly putrescible and a good sand effluent undoubtedly stable; but in that class of effluents in which the chemical analysis yields indefinite information, and in which the putrescibility test is most valuable, the final result is more or less dependent upon the particular technique employed.

*Historical.*—The most distinctive phenomenon associated with putrefaction is the production of offensive odors. These are due to various substances, generally gaseous, such as ammonia and the higher amido compounds, sulphides, mercaptans, and many others, all of which are associated as a rule with the anaerobic decomposition of proteid matter. It was therefore natural that the earliest tests employed to determine putrescibility should be based upon the development of the odors of these bodies shown in a sample kept in an air-tight vessel for a period of a few days. Such was the test originally devised by Scudder.<sup>1</sup> A glass stoppered bottle was filled completely with the sewage, and stored at a temperature of about 27°C. for several days. If it became dark colored and when opened gave off putrefactive odors it was considered putrescible. This so-called "smell test" was devised and used in 1895. Later, in 1899, at Manchester, the test was placed upon a more scientific footing by making initial and final determinations of the oxygen consumed from permanganate, by the "three-minute cold test;"<sup>2</sup> ferrous salts, hydrogen sulphide, and unsaturated organic bodies which are characteristic of putrefactive changes, are readily and instantly oxidized by permanganate, and hence their presence is readily detected by an increase in the oxygen-consumed figure. Modifications of the test in which the oxygen-consumed figures were determined by longer periods of contact or by boiling with the permanganate, have proved much less satisfactory, since in any such procedure a slight increase is often masked or completely nullified by a simultaneous decrease in the oxidizability of the remaining organic matter.

Later modifications of the test have been made by determining the free dissolved oxygen, and the nitrates and nitrites in the effluent before and after incubation. Such determinations show better than any other the actual course of the changes that are taking place, but the results do not always bear directly upon the question of the stability of the effluent. A marked decrease in these values does, indeed, indicate putrescible or decomposable organic matter; but if, by that decrease, the organic matter be rendered stable before the complete exhaustion of the available oxygen, then it may fairly be said that the effluent as a whole is stable. There are three possible cases. The organic matter of the effluent may be stable or it may be putrescible, i. e., capable of further anaerobic decomposition; in the latter case there may be a sufficient supply of available oxygen, free oxygen, nitrates, and nitrites present to render the organic matter stable, or the amount may be insufficient. Only in this final case is the effluent as a whole putrescible as determined by the older tests. Therefore, in interpreting determinations of oxygen dissolved, nitrates, and nitrites, it is not their diminution but their total exhaustion which indicates putrefaction.

Stoddart<sup>3</sup> made quantitative tests for hydrogen sulphide, and Dunbar and Thumm<sup>4</sup>

<sup>1</sup> *Interim Rep. Royal Commission on Sewage Disposal*, 1901, 2, Question 5988.

<sup>2</sup> FOWLER, *Sewage Works Analysis*, 1902.

<sup>3</sup> *Analyst*, 1901, 26, p. 281.

<sup>4</sup> *Beitrag zum zeitigen Stande der Abwasserreinigungsfrage*, 1902.

tested qualitatively for that gas with lead acetate paper. Both of these modifications, particularly the latter, make the test a much more severe one.

In contrast to such strict standards there has been on the other hand a tendency toward less rigid criteria. Thus Adeney<sup>1</sup> claims that an effluent discharged into a running stream containing itself an abundance of oxygen, should be diluted before testing with the waters of that stream in the proportion of their relative discharges. Clark<sup>2</sup> has used a similar method in a study of the stability of the organic matter in effluents, but as a practical test of the work of a filter it seems altogether too lenient. We have a right to demand that the effluent from a good sewage plant shall not diminish the ratio of oxidizable matter to available oxygen in the stream into which it discharges: by Adeney's test it would pass unless the entire purifying capacity of the stream would be destroyed.

An exhaustive study of the whole subject of putrescibility has recently been made by Johnson, Copeland, and Kimberly.<sup>3</sup> These investigators endeavored to find a relation between the putrescibility or stability of an effluent and its chemical analysis, particularly the oxygen consumed figures on one hand, representing oxidizable material, and the available oxygen in the form of free oxygen, nitrates, and nitrites on the other hand. The data required in establishing such a relation are:

- 1) The available oxygen in the form of free oxygen, nitrates, and nitrites, and
- 2) The "consumed oxygen," this being taken as one-fifth of the oxygen-consumed value as determined by the five-minute boiling method.

With these data the following provisional criteria of putrescibility were found to hold for the experiments in question.

When the consumed oxygen value is equal to or in excess of the dissolved oxygen figure and there are no nitrates or nitrites present the sample will putrefy.

When the consumed oxygen value is equal to or slightly less than the amount of oxygen in the effluent in the form of nitrates, nitrites, and dissolved oxygen the sample may or may not putrefy.

When the consumed oxygen value is less than the oxygen contained in the effluent in the form of nitrates and nitrites, under ordinary conditions the sample will not putrefy.

None of the tests for stability so far reviewed have proved wholly satisfactory. The "smell test" is inexact; and at the other extreme the determination of oxygen consumed, dissolved oxygen, nitrates, and nitrites, is too cumbrous a process for routine work. Furthermore, it does not accurately measure the relation of oxidizable matter to available oxygen since the oxygen consumed by permanganate bears a variable relation to the organic matter which is oxidizable under natural conditions. The "Manchester test" is somewhat more satisfactory, but this too yields abnormal results at times and only divides effluents into two rough classes without distinction as to their relative grade.

<sup>1</sup> *Rep. Royal Commission on Sewage Disposal*, 1901, 2, Question 2306.

<sup>2</sup> *Ann. Rep. Mass. State Board of Health*, 1902, 34, p. 373.

<sup>3</sup> *Jour. Infect. Dis.*, 1906, Supplm. 2, p. 80.

A new putrescibility test, simple in technique, and measuring accurately and delicately the relation between available oxygen and oxidizable matter, has therefore been a desideratum. Such a method appears at last to be at hand in the methylene blue test, first devised by Spitta<sup>1</sup> for the study of stream pollution and later more thoroughly worked up by Spitta and Weldert<sup>2</sup> as a test for sewage effluents. Methylene blue is a commercial dye-stuff of somewhat complex constitution, having the empirical composition  $C_{16}H_{18}N_3SCl$ . It is prepared technically by the oxidation of dimethyl-p-phenyldiamine with ferric chloride in the presence of hydrogen sulphide. The commercial product is a double chloride of zinc and the above compound. It is an extremely sensitive indicator for hydrogen sulphide and other reducing bodies, being decolorized at once in the presence of even small traces: its decolorization by bacteria has been studied by many observers, the principal of whom are cited by Spitta and Weldert.<sup>3</sup> The technique employed is extremely simple. A small portion of an aqueous solution of the dye (in our experiments 1 c.c. of a 0.1 per cent solution) is added to the effluent in a glass stoppered bottle (250 c.c. capacity in our work), and the sample is then incubated either at 20° C. or at 37° C. The blue color of the solution remains practically unchanged during the period of observation until the available oxygen contained in it is used up and putrefactive conditions arise. At this point the dye is reduced and decolorized. The time required for such decolorization is a quantitative measure of the degree of putrescibility of the sample and the retention of the color for a period of one week or more, at 20° C., or of four days at 37° C., may be taken as an indication of its stability. The criticism of Johnson, Copeland, and Kimberly<sup>4</sup> that "substances other than putrescible organic matters, such as sulphide of iron and hydrogen sulphide," discharge the color of methylene blue before the putrescible matters themselves are able to act, does not seem to us well taken, since such substances are present only when the effluent as a whole is in unstable condition.

Spitta and Weldert used both methylene blue and indonaphthol blue in their experiments, but found the latter difficult to prepare and subject to decomposition. With methylene blue, they tested<sup>5</sup> 46 sew-

<sup>1</sup> *Archiv f. Hyg.*, 1903, 46, p. 113.

<sup>2</sup> *Mitt. a. d. königlichen Prüfungsanstalt f. Wasserversorgung zu Berlin*, 1906, 6, p. 161.

<sup>3</sup> *Loc. cit.*

<sup>4</sup> *Loc. cit.*

age effluents of various types, mostly from contact beds and trickling beds, observing the color and odor at intervals, the total period of observation varying from one day to ten. Thirty-two samples failed to decolorize in 12 hours at  $37^{\circ}$ ; and of these only one gave an odor of hydrogen sulphide, and that after two days. The other 14 samples decolorized in periods varying from an hour and a half to nine hours; all of them underwent offensive putrefaction in from 9 to 120 hours. In another series of experiments it was shown that the reduction of the methylene blue always takes place before any odor of hydrogen sulphide became noticeable. In one case the sample was decolorized after three hours and the odor was apparent only after 48 hours. In three other samples decolorization occurred after 18 hours, four days, and four days, respectively, while hydrogen sulphide could not be detected after 10 days.

Both putrefaction and decolorization will naturally vary with the temperature at which the sample is incubated. Spitta and Weldert<sup>1</sup> give the following results, which indicate that putrefaction occurs from two to four times as fast at  $37^{\circ}$  as at the ordinary room temperature.

TABLE 1.  
EFFECT OF TEMPERATURE ON DECOLORIZATION OF METHYLENE BLUE.  
(Spitta and Weldert.)

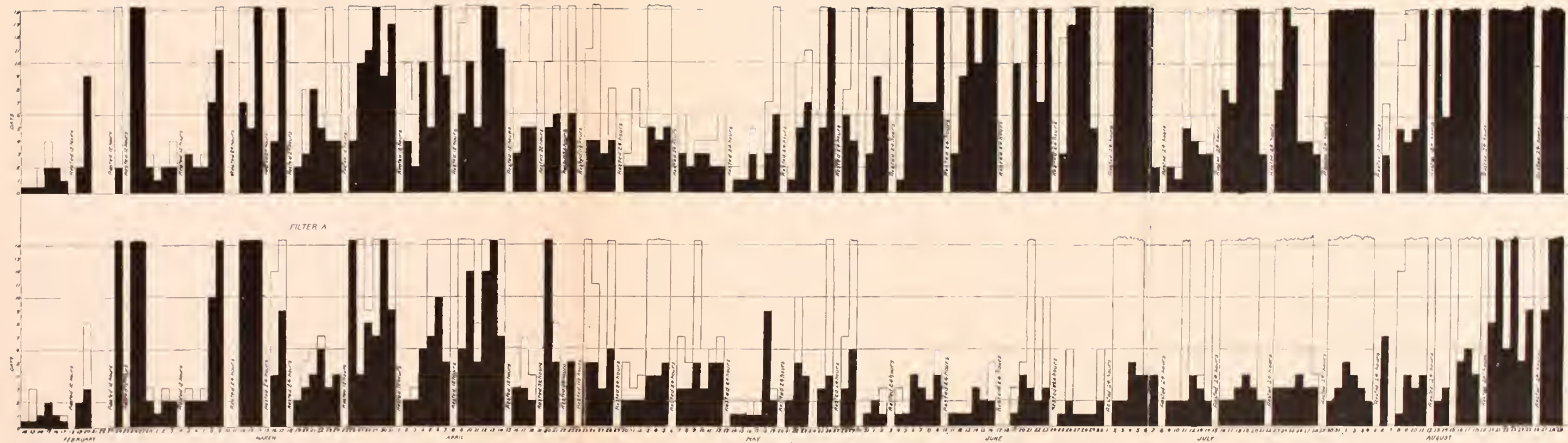
Time of Decolorization in Hours.

Sample	$22^{\circ}$	$28^{\circ}$	$37^{\circ}$
1.....	24	6	6
2.....	3	1.5	1.5
3.....	120	96	48

We have compared the decolorization of 64 samples of trickling filter effluent at  $20^{\circ}$  and  $37^{\circ}$  in order to obtain further light upon this point. Thirty-four of the samples were not reduced after 14 days at either temperature. In 10 cases the  $20^{\circ}$  sample retained its color for 14 days while that kept at  $37^{\circ}$  was decolorized. The period of decolorization at  $20^{\circ}$  was one day in one case (abnormality due evidently to bad sampling), from three to five days in four cases and from five to seven days in five cases. In the other 20 samples the decolorization times for both temperatures were determined to the nearest half-day and the ratios of the  $20^{\circ}$  times to the  $37^{\circ}$  times are tabulated in Table

<sup>1</sup> *Loc. cit.*





Methylene Blue Decolorization Tests. Trickling Filter Effluents. Upper series, filter B, lower series, filter A, Unsettled, Solid blocks, Settled, Outlined blocks. Ordinates, Time of persistence of blue color in days at 20°. Massachusetts Institute of Technology Sewage Experiment Station.



2. The actual number of days varied from half a day to seven days at 20° and from half a day to 13 days at 37° C.

TABLE 2.  
RATIO OF TIME REQUIRED FOR DECOLORIZATION OF TRICKLING FILTER EFFLUENTS AT 20° TO THAT  
REQUIRED AT 37° C.

2.5	2.0	1.6	1.6
1.5	2.6	1.0	0.5
2.7	2.0	4.0	2.2
1.8	2.0	2.0	2.3
1.4	1.9	2.4	2.0

With the exception of two very low ratios (0.5 and 1.0), and one high ratio (4.0), all these figures fall between 1.4 and 2.7 and the average of all is exactly 2.0. It may be considered therefore that methylene blue samples will decolorize at 37° in half the time required at 20°.

It must be remembered in interpreting this table, and all others in which comparative decolorization tests are recorded, that each test must be made in a separate bottle and since the sampling of sewage is never perfect there will be variations due to the chance differences in the several bottles compared. This is particularly true in work like that later cited in Table 5 in which decolorization tests were made in one bottle and analytical data determined on another.

In general it appears from Table 2 that results obtained at 20° bear to those obtained at 37° the ratio of 2 to 1. Stable samples are stable at any temperature. In grading putrescible samples, however, it is evidently necessary to select one temperature and to use it for all comparative work. The temperature of the body has the advantage that it gives results more promptly. Any sample which does not decolorize in four days is certainly stable and a two days' limit would include almost all putrescible effluents. On the other hand, the 20° method, from the very fact that it is slower, permits a more delicate measurement of the relative differences between putrescible effluents of different grades. Decolorization here may occur from the first up to the 14th day. Furthermore, the test can be made, if necessary, at room temperature without an incubator or special apparatus; and this simplicity would be of the greatest importance in small sewage plants without a laboratory or expert supervision. Slight variations from 20° will not introduce a serious

error in the test; and diffuse daylight does not appreciably interfere with it. A series of control experiments, made in daylight, in a box at the room temperature (in summer), and in an incubator at 20°, are recorded in Table 3 and indicate that the test could be made in an ordinary room with fairly controlled temperature. Experiments made during cold weather in a small frame building heated by a stove showed marked abnormalities, on the other hand, and emphasize the fact that larger temperature variations cannot be allowed to go too far.

TABLE 3.  
EFFECT OF TEMPERATURE AND LIGHT ON METHYLENE BLUE TEST.  
Average Time of Decolorization in Days.

WEEK ENDING	EFFLUENT A			EFFLUENT B			EFFLUENT C		
	Summer Temperature		20°	Summer Temperature		20°	Summer Temperature		20°
	Daylight	Dark	Dark	Daylight	Dark	Dark	Daylight	Dark	Dark
June 27.....	1	2	2	11	11	7	2	3	3
July 2.....	3	3	3	12	12	10	3	3	4
July 9.....	2	2	2	2	3	2	2	3	3
July 16.....	2	3	2	9	12	10	13	13	13
July 23.....	2	3	4	7	8	7	14+	14+	14+
July 30.....	2	5	6	13	14+	13	12	14+	13
August 6.....	2	3	3	6	6	6	14+	13	13
August 13.....	3	5	4	13	13	12	13	13	13
August 20.....	10	5	5	14+	14+	14+	14+	14+	14+

With regard to the significance of the methylene blue test in relation to the specific chemical changes which go on in putrefaction, Spitta and Weldert have little to say beyond pointing out that decolorization takes place only after all the available oxygen has been exhausted. This they illustrate by a single experiment, cited in Table 4.

TABLE 4.  
OXYGEN CHANGES IN A TRICKLING FILTER EFFLUENT.  
(Spitta and Weldert.)

Days.....	$\frac{1}{2}$	$\frac{1}{2}$	1	2	3	4	5	10
Dissolved oxygen; c.c. per L.....	2.62	1.44	0.50	0.37	....	0.00	....	0.00
Nitrogen as nitrites and nitrites: Mg. per L	10.0	10.0	10.0	....	....	8.0	....	4.0

The methylene blue remained unchanged up to this point.

We have thought it important to study the relation between decolorization and chemical processes somewhat more fully; and the



results of the examination of five samples of trickling effluent are shown in Table 5.

TABLE 5.  
OXYGEN CHANGES IN PUTRESCIBLE TRICKLING FILTER EFFLUENTS.  
Decolorization of Methylene Blue Indicated by \*.  
(PARTS PER MILLION.)

SAMPLE	DAYS AT 37°	DISSOLVED OXYGEN	NITROGEN AS		OXYGEN CONSUMED 15 MINUTES COLD
			Nitrates	Nitrites	
1.....	Initial	8.0	3.0	1.0	5.0
	1*	0.0	0.0	0.3	5.0
	2	....	....	0.1	....
	Initial	10.0	3.0	1.0	4.0
	1	1.0	3.0	1.0	4.0
	2	0.0	0.0	1.0	3.4
	3	0.0	...	0.1	4.0
	4	...	...	0.08	4.0
	5	...	...	0.03	3.8
2.....	7	...	...	0.00	4.0
	8	...	...	...	4.8
	9	...	...	...	6.3
	Initial	8.0	3.5	0.5	6.0
	1	1.0	3.0	1.2	4.8
	2	1.2	3.0	1.4	5.7
	3	0.4	3.0	1.0	5.2
	4	0.0	0.0	0.0	5.0
	7*	...	...	...	...
3.....	Initial	10.8	4.0	0.3	4.2
	1	1.8	3.0	0.3	4.5
	2	0.2	3.0	0.5	4.4
	3	0.2	3.0	0.1	3.4
	4	0.6	2.5	0.1	2.5
	5	0.0	1.0	0.1	4.3
	6	...	0.0	0.05	3.6
	7	...	0.0	0.0	2.7
	Initial	9.2	3.0	0.6	5.2
4..... M. B. sample lost	1	1.2	2.5	0.6	5.3
	2*	0.0	0.0	0.0	5.3
	3	0.0	0.0	0.0	5.7
	4	...	...	...	4.6
	5	...	...	...	3.9
	6	...	...	...	6.1
	7	...	...	...	6.9
	Initial	9.2	3.0	0.6	5.2
	1	1.2	2.5	0.6	5.3
5.....	2*	0.0	0.0	0.0	5.3
	3	0.0	0.0	0.0	5.7
	4	...	...	...	4.6
	5	...	...	...	3.9
	6	...	...	...	6.1
	7	...	...	...	6.9
	Initial	9.2	3.0	0.6	5.2
	1	1.2	2.5	0.6	5.3
	2*	0.0	0.0	0.0	5.3

It is evident that the free dissolved oxygen is absorbed first by the organic compounds present; then the nitrates disappear, and finally the nitrites; and the methylene blue is generally attacked just after the disappearance of the latter. Sometimes, as in sample 3, there is a considerable delay between the disappearance of nitrites and the decolorization of the methylene blue. This may probably occur when the organic matter and the available oxygen are about evenly balanced, so that after the exhaustion of the oxygen putrefactive changes are set up only somewhat slowly. Whenever the methylene blue is decolorized, it may be assumed that all oxygen as nitrates or nitrites has disappeared; and vice versa, when the blue color is retained, traces of these bodies are generally present. This

condition is illustrated in Table 6 for trickling filter effluents which did not decolorize after 14 days at 37°. It will be noticed that these effluents contained a considerable amount of organic matter as measured by oxygen consumed and that in all cases there was a

TABLE 6.  
OXYGEN AND OXIDIZABLE MATTER IN STABLE TRICKLING FILTER EFFLUENTS.  
(PARTS PER MILLION.)

INITIAL COMPOSITION				AFTER 14 DAYS AT 37°			
Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold	Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold
	Nitrites	Nitrates			Nitrites	Nitrates	
6.8	4.0	5.5	5.6	0.0	0.0	0.0	3.2
2.8	3.0	5.5	8.4	0.3	3.5	1.5	7.6
5.2*	0.0	15.0	4.4	4.0	0.1	15.0	4.4
7.2	5.0	3.0	18.8	0.1	0.4	1.5	8.9
4.4	3.0	3.0	5.2	0.0	0.1	1.5	3.5
7.2	4.0	6.0	16.8	0.1	0.5	5.0	15.8
4.6	3.0	6.0	10.4	0.0	1.5	1.0	12.2
4.4	2.5	5.5	9.6	0.0	0.0	0.0	7.0
4.4	3.0	5.5	8.4	0.2	0.8	1.5	2.0
6.6	1.0	2.0	17.4	0.0	3.0	0.1	2.6
4.2	1.5	3.0	7.2	0.0	1.0	0.0	0.6
5.7	2.5	3.0	8.4	0.0	0.0	0.0	5.2
4.3	3.0	4.0	7.0	0.6	6.0	2.0	7.7

\* Sand filter effluent.

marked reduction in oxygen content during incubation. Nitrites and nitrates remained, however, in all but three cases.

This condition may be compared with that of the putrescible effluents in Table 5 and with four other sets of analyses quoted in Table 7.

TABLE 7.  
OXYGEN AND OXIDIZABLE ORGANIC MATTER IN PUTRESCIBLE TRICKLING FILTER EFFLUENT.  
(PARTS PER MILLION.)

INITIAL COMPOSITION				AGE, HOURS AT 37°	AFTER DECOLORIZATION			
Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold		Dissolved Oxygen	Nitrogen as		Oxygen Consumed 15 Min. Cold
	Nitrites	Nitrates				Nitrites	Nitrates	
4.3	2.0	3.0	24.4	24	0	0	1.5	25.2
1.1	2.5	3.0	14.0	12	0	0	0.0	14.0
5.8	1.5	5.0	15.6	40	0	0	0.0	16.0
3.8	1.5	5.0	8.8	120	0	0	0.0	2.8

An interesting practical question is the relation of the methylene blue test to the original constitution of the effluent as determined by chemical analysis. If our methods were sufficiently precise we should

be able to predict from determinations of organic matter and oxygen just what the result of their interaction will be. The condition of an effluent, as indicated by the relation of "oxygen consumed" to available oxygen, corresponds, however, to its putrescibility only roughly. Johnson, Copeland, and Kimberly from their investigations made the generalizations previously quoted as to the relation between the production of odors and the analytical data; but their tables offer numerous exceptions to this general rule. Our experience with the methylene blue test has been much the same. Comparing Tables 5, 6, and 7, a general difference between the stable and putrescible samples is apparent, the former, in Table 6, having on the whole a considerably higher ratio of available oxygen to "oxygen consumed" than the latter as shown in Tables 5 and 7. There are, however, many samples which contradict this general rule, and we have been unable on any basis of calculation to find a marked and constant difference in analysis between stable and putrescible samples. This is still more forcibly brought out in Table 8 which represents the average results of determinations carried on for six months, the analytical data being determined weekly on composite samples, and the methylene blue test made six times a week. The methylene blue samples were kept for 14 days and those which retained their color for that time would probably have done so indefinitely. In calcu-

TABLE 8.  
MONTHLY AVERAGE ANALYSES OF TRICKLING FILTER EFFLUENTS AND AVERAGE DECOLORIZATION TESTS.

EFFLUENT, FILTER A.					
MONTH	OXYGEN CON- SUMED 30 MIN. BOIL	NITROGEN AS		DISSOLVED OXYGEN	METHYLENE BLUE TEST DAYS AT 20°
		Nitrites	Nitrates		
March.....	47	0.6	3.6	8.8	7
April.....	54	0.5	4.6	4.0	7
May.....	59	1.1	4.5	6.0	3.5
June.....	40	4.0	3.6	4.4	3
July.....	43	2.5	3.8	5.0	2.5
August.....	34	2.5	4.0	4.7	5

EFFLUENT, FILTER B.					
March.....	28	0.0	4.1	7.5	6
April.....	41	0.2	5.1	3.8	8
May.....	46	1.2	4.4	5.1	4
June.....	31	2.7	3.8	4.1	10
July.....	36	2.8	5.3	4.8	10
August.....	33	3.5	4.0	5.8	11

lating averages they receive only the value of 14 days, however. The monthly averages of 10 days or more represent effluents of high stability and those of three and four days, effluents which were distinctly putrescible.

Evidently the methylene blue test does not correspond strictly to our analytical data. There are no differences in the oxygen and "oxygen consumed" values sufficient to account for the wide difference in stability between the effluents for Filter A for June and Filter B for April, for example, or between Filters A and B for August. It is evident that certain factors affect the stability of an effluent which are not revealed by the ordinary methods of sanitary chemistry. These factors must be taken into account in judging of the success of a sewage disposal plant, since the stability of the effluent is precisely the point of greatest importance.

The methylene blue test, during a year's use in the routine study of the trickling filters at the Sewage Experiment Station, has proved itself admirably adapted to the detection of slight variations in this crucial quality of stability. The general results of the test as shown in diagrammatic form on the appended folded sheet, indicate quite clearly its value. Filter A was a trickling filter 8 ft. deep and 10'  $\times$  10' in area, filled with crushed stone from 1½" to 2" in diameter and receiving crude Boston sewage at a rate of two million gallons per acre per day. Filter B was exactly similar but was dosed with the effluent from a septic tank of seven hours' capacity. The block diagrams refer in each case to the effluents as they flowed from the filters; and the outline diagram to the same effluents settled for two hours in tanks of a modified Dortmund pattern. It will be noticed that the two filters gave similar results during the first winter, the unsettled effluents being generally putrescible and the settled effluents generally stable. In April both fell off somewhat, but in May the filter taking septic sewage improved in operation and all through the summer gave effluents which even without sedimentation were generally stable. Filter A, receiving crude sewage, continued to deteriorate. Through June even its settled effluent usually putrefied and in July and August its effluents were stable only after sedimentation. Here were radical differences in the work of two filters, so radical that one might be considered a failure and the other a success. Yet



the analytical data for these same filters, as given in Table 8, offered no adequate hint of the distinction.

We believe, therefore, that some special test for stability is urgently needed in all sewage analysis; and the methylene blue test seems to us to have certain special advantages, and to deserve wider attention than it has so far received. It directly measures the quality of most importance in a sewage effluent, its freedom from the tendency to putrefactive change. It registers this with a greater delicacy than any method with which we are acquainted. The range between an effluent which decolorizes in 24 hours and one which retains its color for two weeks makes it possible to distinguish a dozen grades of effluents between the highly putrescible and the certainly stable one. The effect of the Sunday resting-period in decreasing the stability of the effluents shown on the diagram is a striking instance of the way in which this test reveals slight derangements of a delicately organized filter. If results are desired promptly the test may be made at 37° and will then distinguish between a putrescible and a stable effluent in two days. It is so simple in technique as to be peculiarly adapted to sewage works where neither a laboratory nor an expert chemist are available.

# THE APPLICATION TO A SOFT-WATER SEWAGE OF DIRECT PROCESSES FOR THE DETERMINA- TION OF KJELDAHL NITROGEN AND NITROGEN AS FREE AMMONIA

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WHEN the Experiment Station at Waterbury, Connecticut, was started in operation, December, 1905, it was decided to adopt direct processes for the determination of Kjeldahl nitrogen and nitrogen as free ammonia.

After reviewing the literature on the direct determination of Kjeldahl nitrogen, it was decided to apply the method described by Kimberly and Roberts,<sup>1</sup> and used at the Columbus Sewage Testing Station. In carrying out this method under Waterbury conditions, it was soon found that several points of moment under Columbus conditions did not affect the applicability of the method for this soft-water sewage, and hence it was possible to simplify the technique. It is the purpose of the writer to outline briefly the experiments at Waterbury with direct nesslerization in Kjeldahl nitrogen work, together with a brief discussion of the applicability of the standard method for the direct determination of nitrogen as free ammonia, in each instance showing by comparative data the relation between results by direct and distilled methods.

## THE DIRECT KJELDAHL PROCESS.

In developing the direct Kjeldahl procedure for the soft-water sewage of Waterbury, attention was directed to the question of the interference of calcium and magnesium, which Columbus experience indicated to be the controlling feature in successful direct nesslerization. Analysis showed, however, that neither calcium nor magnesium was present in the Waterbury sewage to such an extent as to cause turbidity in a nesslerized tube.

After eliminating from the discussion the calcium and magnesium factors, the next step was to determine the effect of different grades

<sup>1</sup> *Jour. Infect. Dis.*, Supplm. 2, 1906, p. 109.

of caustic soda upon the color produced by Nessler's reagent; for, as the Columbus results indicated, the purity of the caustic soda used in neutralizing the acid digestates controls in a large measure the clearness of ammonia tubes when nesslerized.

Caustic soda from sodium was tried first and with highly satisfactory results, but the cost seemed too high for routine laboratory work. Caustic soda by alcohol was next used. No cloudiness resulted, but, with increasing quantities of the alkali, the depth of the color produced increased slightly, as has been found by former experience.<sup>1, 2</sup>

The grade of caustic soda commonly known as "Purified Sticks" did not give satisfactory results. When fully purified the "blank" was high and the amount of preparatory treatment necessary was not commensurate with the difference in cost between this product and that by alcohol.

#### THE WATERBURY METHOD.

Having satisfactorily disposed of the question of turbidity and that of the most suitable grade of caustic soda, our attention was directed so to modify details of the method used at Columbus as to develop a process which would be practical and reliable under Waterbury conditions. After some experimentation the method finally assumed the following definite form.

*Method.*—A suitable portion of the sample (50 to 100 c.c.) is placed in a 300 c.c. Kjeldahl flask, diluted to a volume of 150 c.c. and boiled down to 25 c.c. to insure the removal of the nitrogen as free ammonia. The usual amount of sulphuric acid is then added, and the digestion is then carried to completion. After cooling, the contents of the flask are removed into a 100 c.c. graduated flask, thoroughly shaken, and from the sample there are removed 50 c.c. and placed into a second 100 c.c. flask. The caustic soda is now added, just short of the neutral point; the flask is cooled and the digestate made alkaline, adding in excess not more than 1 c.c. of a 25 per cent caustic-soda solution. The solution is then poured into a dry four-ounce bottle which is tightly stoppered, and set aside for twenty-four hours. During this period it is advisable to agitate the bottle slightly, so as to detach any suspended particles that may cling to the sides.

At the end of 24 hours, or at least of 12 hours, 10 c.c. of the clear supernatant liquor are then removed and placed into a 50 c.c. Nessler tube to which there has been previously added about 25 c.c. of ammonia-free water.

The tubes are next filled to the mark and are mixed by inverting three or four times; the tubes are then nesslerized and read in the usual manner.

<sup>1</sup> NESSLER, *Tiemann-Gartner's Handbuch*, 1905, p. 114.

<sup>2</sup> HAZEN AND CLARK, *Amer. Chem. Jour.*, 1890, 12, p. 425.

To compute the results, which, of course, are in terms of organic nitrogen, it is convenient to use the formula worked out by Kimberly and Roberts:<sup>1</sup>

$$\frac{(N \times 20) - \text{Blank} \times 1,000,000 \times .00001}{S};$$

where N = c.c. standard reading, S = c.c. sample digested.

#### DISCUSSION OF DETAILS OF METHOD.

In the Columbus method no provision was made for first removing the free ammonia before the digestion. In applying the Columbus method to the work at Waterbury, it was not found practicable to obtain results comparable with those of the distillation process, nor with each other, for the probable reason that the quantity of sample taken for analysis, especially in the case of strong sewages, was so small as to introduce a large factor of error. To avoid this, it was decided to remove by boiling the nitrogen as free ammonia, thus permitting the use of a greater quantity of the sample. In the opinion of the writer, the necessity for the removal of the nitrogen as free ammonia before digestion, is probably controlled in a large measure by the relative amounts of nitrogen in that form and as organic nitrogen, although he favors the complete removal of the nitrogen as free ammonia.

It was found important to measure accurately the quantity of caustic soda to be used in neutralizing the acid digestates. For this purpose it was found convenient to arrange a four-liter bottle of the caustic soda in such a position that the solution could be blown into a burette whereby the amount of alkali could be accurately measured.

The necessity for using in excess not more than 1 c.c. of a 25 per cent caustic-soda solution lies in the fact that tubes which contain a large excess of alkali do not mix well, on account of the difference between the specific gravity of the dilution water and that of the alkali; and further because samples which are barely alkaline never become entirely free from suspended particles. According to the writer, a failure to observe either one of the precautions is sure to result in cloudy tubes.

#### COMPARISON OF RESULTS BY DIRECT AND DISTILLED METHODS.

To judge of the feasibility of adopting the direct process, as a routine procedure, a large number of analyses were made, comparing

<sup>1</sup> *Jour Infect. Dis.*, Suppl. 2, 1906, p. 117.



the results of the direct process with those of the distilled, as obtained according to the standard procedure. Representative analyses are listed below, and a study of the results indicates that the direct method is capable of affording very satisfactory results, which agree closely with the results of the distillation method.

TABLE I.

EFFICIENCY OF DIRECT PROCESS FOR DETERMINING KJELDAHL NITROGEN AS COMPARED WITH THE DISTILLATION METHOD.

SOURCE OF SAMPLE	ORGANIC	
	Direct	Distilled
Crude sewage.....	7.0	7.0
	7.3	7.4
	7.0	6.9
Septic sewage .....	18.0	19.0
	16.4	16.3
	11.5	12.2
Sprinkling filter effluent .....	15.0	14.8
	10.7	11.2
	8.7	8.4
Effluent contact filter .....	12.0	12.0
	10.5	11.0
	9.8	9.5
Sand filter effluent.....	7.8	8.0
	5.2	5.0
	9.0	9.4

#### APPLICATION OF THE DIRECT KJELDAHL PROCESS TO THE DETERMINATION OF NITROGEN IN SEWAGE SLUDGE.

Following the general scheme outlined for the analysis of samples of sewage and effluent, the direct Kjeldahl process was applied to sludge analysis. The procedure adopted was as follows:

An amount of sample is taken that will yield from 0.005 to 0.02 gm. of nitrogen; usually about 0.5 gm. of dried sludge lies within the above limits. Twenty c.c. of sulphuric acid are then added and the digestion continued until the digestate becomes clear. After cooling, the contents of the flask are rinsed into a 250 c.c. graduated flask, and made up to the mark with ammonia-free water. Twenty-five c.c. of this solution are next introduced into a 200 c.c. flask made slightly alkaline with caustic soda, exercising precautions similar to those necessary in sewage work, the solution made up to 200 c.c. poured into an eight-ounce bottle and allowed to stand for 24 hours. Five c.c. of the clear supernatant liquid are then removed and nesslerized in the usual way.

Very satisfactory results have been obtained by this method, as indicated by the table below in which there are presented the results of duplicate analysis of sewage sludge.

TABLE 2.  
REPRESENTATIVE DUPLICATE RESULTS OF NITROGEN DETERMINATION IN SEWAGE SLUDGE.

SOURCE OF ANALYSES	DUPLICATE ANALYSES	
	Per Cent Nitrogen	Per Cent Nitrogen
Grit chamber sludge.....	4.74 1.66 1.15	4.50 1.24 1.34
Settling tank sludge.....	2.37 2.04 1.98	2.40 1.85 2.10
Septic sludge.....	1.60 1.60 2.00	1.60 1.60 1.97

NOTES ON THE DIRECT DETERMINATION OF FREE AMMONIA BY THE  
STANDARD METHOD.

In determining nitrogen as free ammonia by a direct process, the method outlined by the Committee on Standard Methods of the Public Health Association has been followed, except that the sewage is filtered before the addition of the caustic soda and the copper sulphate.<sup>1, 2</sup>

*Necessity for filtration.*—The idea of a preliminary filtration of the sample was suggested by the fact that when the unfiltered sewage was treated there remained in suspension from 30 to 50 parts of colloidal matter, as against from 10 to 15 parts, in case a preliminary filtration was included in the technique.

From experience gained during the work at Waterbury it has been found that in case the supernatant liquid, from a direct nitrogen as free ammonia determination, contains more than 30 parts per million of colloidal matter; high results will be obtained even though but 5 c.c. of the supernatant are removed and then diluted 10 times. As above mentioned, this feature is entirely eliminated by passing the sample through filter paper before beginning the determination.

*Entrainment.*—In all direct processes there is of course afforded an opportunity for slightly low results, owing to the entrainment of ammonium salts by the forming of precipitates. In agreement with the results obtained by other investigators,<sup>3</sup> the Waterbury results

<sup>1</sup> FARENSTEINER, BUTTENBURG, AND KORN, *Leitfaden f. d. chem. Untersuch. von Abwässer*, Hamburg, 1902

<sup>2</sup> PHELPS, "Determination of Ammonia in Sewage," *Jour. Infect. Dis.*, 1904, 1, p. 327.

<sup>3</sup> KIMBERLY AND ROBERTS, *Jour. Infect. Dis.*, Suppl. 2, 1906, p. 119.

indicate that the nitrogen lost by this means may be considered a negative quantity. This, in the opinion of the writer, may be inferred from the table comparing the results of Kjeldahl nitrogen determinations by direct and distilled processes.

As evidence to support the view that the entrainment factor is by no means of practicable moment, there is presented below a table showing comparative results of nitrogen as free ammonia determined by the direct and distillation processes respectively.

TABLE 3.  
RESULTS OF NITROGEN AS FREE AMMONIA BY DIRECT AND BY THE DISTILLATION PROCESSES

Source of Sample	Direct	Distilled
Crude sewage.....	17.5	18.0
	10.3	10.3
	17.0	16.8
Septic sewage.....	12.0	12.0
	9.5	9.4
	9.8	8.9
Sprinkling filter effluent.....	6.2	5.8
	6.1	6.1
	5.9	5.6
Sand filter effluent.....	2.5	2.0
	3.8	4.5
	2.4	2.4

#### CONCLUSIONS.

In conclusion it may be said that the direct process for the determination of Kjeldahl nitrogen, and that for the determination of nitrogen as free ammonia, have been entirely satisfactory under Waterbury conditions. Out of some 4,000 or 5,000 analyses, not more than 10 per cent of the determinations have been in error, due to cloudy nesslerized tubes, and in almost every case the cause of turbidity could be traced to some error of technique and not to any inherent fault in the process itself. Furthermore, by adopting the above methods it has been possible to increase the number of samples analyzed, and to obtain just as efficient results as if the distillation method had been used.

Acknowledgments are here given to Mr. William Gavin Taylor, Resident Engineer, for the many courtesies extended the writer during the preparation of this paper and especially to Mr. R. A. Cairns, City Engineer, Waterbury, Connecticut, for permitting the printing of these studies before the appearance of the Experiment Station Report.

# EXPERIMENTS WITH METHODS FOR THE RAPID DETECTION OF GELATIN LIQUEFACTION IN THE DETERMINATION OF *B. COLI*.

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IN determining the presence or absence of bacteria of the colon type by the methods approved by the Committee on Standard Methods of Water Analysis, the procedure consists essentially of three steps: first, a detection of gas-producing organisms by means of a fermentation test; second, the separation of acid-fermenting types from other types by plating on litmus-lactose agar and the transfer of typical colonies to agar streaks; third, the identification of the cultures so isolated by their cultural and biochemical reactions when grown in dextrose broth, milk, nitrated peptone solution, Dunham's solution, and gelatin. Since cultures of the colon type grow rapidly and luxuriantly at 40° C., it is possible to complete the first two steps and the identification tests for fermentation, milk coagulation, and nitrate reduction within four days, and the indol test within seven days from the time that the examination of the sample was begun. Since the ordinary culture gelatin does not remain solid at temperatures as high as 40° C., and as the liquefaction of gelatin proceeds very slowly in the case of many common types of bacteria, which can best be excluded from the colon type by this test, it is customary to incubate the gelatin cultures at 20° C. for a period of 14 days. The delay of 10 days required to obtain the liquefaction tests after the other tests are complete is objectionable in many cases where it is desirable that analyses should be reported at the earliest possible date, and many methods have been proposed by which this delay might be obviated. A number of these methods, all based upon the assumption that because the types of bacteria commonly encountered in testing for *B. coli* grow more rapidly and more luxuriantly at body temperature than at room temperature the production of proteolytic enzymes will be correspondingly rapid at the higher temperature, have been investigated at the Lawrence Experiment Station during the past eight



years. Since data which tend to disprove this fundamental assumption must apply with equal force to all the methods tested, statistics regarding only one of the methods will be presented, together with a general statement of the results obtained in tests of the other methods. It is not the purpose of the present paper to enter into a general consideration of the properties of bacterial enzymes, or the conditions under which those properties are manifest, except in so far as they may apply to the specific problem of obtaining a rapid diagnosis of gelatin liquefaction in routine *B. coli* tests.

*Substitutes for gelatin.*—The three media commonly used for liquefaction tests in bacteriology are gelatin, blood serum, and casein. At the suggestion of the late Dr. Wyatt Johnston, the writer studied the possibility of substituting liquefaction tests on Löffler's blood serum at body temperature for gelatin tests at room temperatures some years ago. In that investigation it was found that a considerable percentage of cultures liquefying gelatin in 14 days at 20° C. failed to liquefy serum at 40° C. in four days or less, although a luxuriant growth usually occurred in 18 to 24 hours. In an investigation of the species of bacteria commonly found associated with *B. coli* at Lawrence, out of 46 different species isolated and studied, six only were found which liquefied both gelatin and serum, while 12 liquefied gelatin but did not liquefy serum, and five liquefied serum but did not liquefy gelatin. In other words, we would have obtained a correct report with only 13 per cent of the species, from liquefaction tests on serum, when the tests were made at 20° C., and it is quite probable that a shorter incubation at 40° C. would have shown even a smaller percentage accuracy.

The great difficulty in preparing a casein medium upon which liquefaction tests can be accurately made is a serious objection to its use. No direct investigation of casein as a substitute for gelatin has been made at Lawrence. In the study of species of bacteria mentioned above, however, only five species liquefied both casein and gelatin, and 13 species which liquefied gelatin failed to liquefy casein.

The investigations of Malfitano,<sup>1</sup> published in 1903, definitely established the fact that the liquefaction of albumen and of gelatin

<sup>1</sup> *Compt. rend. de la Soc. de Biol.*, 1903, 55, p. 843.

was due to entirely separate and distinct enzymes, and show the futility of attempting to substitute serum or casein for gelatin.

*Modified gelatin.*—A number of processes for preparing a gelatin medium which should remain solid at 40° C. and at the same time embody the properties of the ordinary culture gelatin have been tried.

Mixtures of gelatin and agar were first proposed by Hiss<sup>1</sup> for separating the typhoid, colon, and paracolon types. Attempts to use media of this type for liquefaction tests at 40° C. proved unsuccessful for the reason that if the proportion of agar present was great enough to make the media even reasonably stiff, liquefaction occurred very slowly or not at all, while a fluid or semifluid mixture had no advantages over gelatin alone.

Mixtures of gelatin and serum were suggested by the gelatin-agar mixtures. A number of such mixtures were tried with little success, cultures which liquefied gelatin at 20° C. frequently failing to show any indication of liquefaction when grown on these media for four days at 40° C. Any liquefaction results obtained on such media would, of course, be subject to the same inaccuracy as in the use of serum alone.

In 1901 a German investigator<sup>2</sup> suggested that the melting-point of gelatin might be raised to above 40° C. by the addition of small quantities of formalin without impairing its value as a medium for the cultivation of bacteria. Many attempts have been made at Lawrence to prepare a satisfactory medium in this manner. The problem is to add just the proper amount of formalin. If too much be added the gelatin becomes very hard and liquefaction proceeds very slowly, any considerable excess of formalin will effectually prevent the development of bacterial growth, while if too little be added the melting-point will not be raised sufficiently to prevent the gelatin becoming fluid at 40° C. The process might possibly be worked on a commercial scale to produce a bacteriological gelatin, but its application in the laboratory appears to be impractical.

Attempts to prepare gelatin media with a melting-point between 40° and 50° C. by the addition of alcohol or tannic acid were also attended with little success, the difficulties encountered being similar to those in the formalin process.

<sup>1</sup> *Jour. Med. Res.*, 1902, 8, p. 148.

<sup>2</sup> *Centralbl. J. Bakt.*, 1901, 30, Abt. 1, p. 368.

*Preliminary cultivation methods.*—The small success obtained in the experiments previously described led to a radical change in procedure. Cultures of liquefying bacteria were seeded into broth and incubated 24 to 48 hours at 40° C., 1 c.c. of the luxuriant culture so obtained was mixed with 5 c.c. of fluid gelatin, and, after solidifying the gelatin, the culture was incubated at 20° C. By such procedure it was hoped that the introduction of a large number of bacteria, together with whatever enzymes might have been formed in the broth, would result in liquefaction becoming manifest in a shorter period than would be the case when stab cultures were used.

The conclusions of Brunton and Macfadyen,<sup>1</sup> that enzymes produced in meat broth liquefied gelatin more rapidly than did those produced in gelatin itself, would lend credence to the advisability of such a procedure.

Cultures started in this way quite uniformly liquefied one to two days earlier than did stab cultures made at the time that the broth cultures were mixed with the gelatin, showing that greater bacterial activity had resulted from the introduction of large numbers of bacteria into the gelatin, but the time required to prepare the "starter," together with the time required to obtain the liquefaction test in the enriched gelatin culture, was about the same as that required for a similar test with stab cultures direct.

A modification of the preceding method consisted in incubating a broth culture of the test organism for four days at 40° C. and then testing for the presence of liquefying enzymes by pouring the culture on the surface of gelatin after marking the level of the gelatin surface, determining whether liquefaction ensued by any change in the depth of the gelatin. This method proved to be less efficient as a means of rapid liquefaction diagnosis than was the preceding one.

Fermi<sup>2</sup> states that the production of gelatinase is more abundant in gelatin media than in any other media and his statement was confirmed by Abbott and Gildersleeve.<sup>3</sup> In the recent experiments of Jordan<sup>4</sup> it was found that the rate of enzyme production during the first 10 days with seven different rapidly liquefying species, was more

<sup>1</sup> *Proc. Roy. Soc.*, 1889, 46, p. 543.

<sup>2</sup> *Centralbl. f. Bakt.*, 1890, 7, p. 469.

<sup>3</sup> *Jour. Med. Res.*, 1903, 10, p. 42.

<sup>4</sup> *Biological Studies by Pupils of Wm. T. Sedgwick*, University of Chicago Press, 1906, p. 127.

rapid in broth than in gelatin at 37.5° C. in four out of seven experiments, while it was more rapid in broth at 20° C. in only two out of seven experiments.

*Gelatin at 20° and 40° C.*—The results obtained in the preceding experiments having shown that it was futile to attempt to obtain early liquefaction results by cultivation in broth, studies were commenced in which gelatin cultures were incubated at 40° C., the gelatin of course being fluid at that temperature. After some preliminary experiments, a routine procedure was adopted as follows: Cultures which had shown the power to liquefy gelatin at 20° C. during the regular *B. coli* tests, were transferred to agar streaks, grown over night at 40° C., and were then reinoculated into gelatin tubes and placed in the 40° C. incubator for four days. The fluid gelatin cultures so obtained were removed at the end of 24 hours, two days, three days, and four days, and placed on ice for one hour, after which treatment those tubes in which the gelatin had solidified were returned to the incubator and the cultures in tubes in which the gelatin failed to solidify in one hour on ice were recorded as having produced liquefaction.

The investigation extended over a period of 10 months ending November 1, 1903. During this time about 2,800 cultures obtained in routine *B. coli* determinations from samples from a variety of sources were submitted to the gelatin liquefaction test by the standard procedure, and 368, or 13 per cent of these cultures, were found to show signs of liquefaction in 14 days or less at 20° C.; 288 cultures, selected at random from among these liquefying cultures, were tested for liquefaction at 40° C. by the method described above. In the routine liquefaction tests at Lawrence the approximate time required for liquefaction to ensue is shown by readings made on the fourth, seventh, tenth, and fourteenth days, and the type of liquefaction has always been recorded, three primary types of liquefaction being recognized: the stratiform type, the funnel, including the saccate and infundibuliform types, and the cup, including the craterform and the napiform types. The detailed results of the tests at 40° C. are shown in Table 1, the cultures being grouped according to their variations at 20° C.

Of the 288 cultures tested by both methods liquefaction developed



TABLE 1.  
RESULTS OF TESTS FOR LIQUEFACTION AT 20° C. AND AT 40° C.

GELATIN AT 20° C.			GELATIN AT 40° C.					
Type of Liquefaction	Date Liquefaction Was First Noted	Number of Cultures	Number of Cultures First Showing Liquefaction on				Total Number of Cultures	
			First Day	Second Day	Third Day	Fourth Day	Liq.	Non-Liq.
Cup.....	4th day	0	3	2	1	0	6	3
	7th	6	1	0	0	0	1	5
	17	3	0	0	0	0	3	14
	10th	121	3	0	0	4	22	99
Funnel.....	14th	48	18	0	0	0	36	12
	4th	33	1	1	1	1	0	10
	7th	10	0	0	0	0	3	14
	10th	17	2	1	0	0	9	22
Stratiform.....	14th	31	6	1	0	2	16	5
	4th	21	12	3	1	0	1	2
	7th	3	1	0	0	0	2	0
	10th	2	1	0	1	0	0	3
	14th	3	0	0	0	0	0	

at 20° C. in 27 per cent in four days or less, in 7 per cent between the fourth and seventh days, in 12 per cent between the seventh and tenth days, and in 54 per cent between the 10th and 14th days, 53 per cent of the liquefactions being of the cup type, 37 per cent of the funnel type, and 10 per cent of the stratiform type.

The rate of liquefaction at 20° C. by cultures producing the cup type of liquefaction was very different from that of cultures producing the funnel and stratiform types, 73 per cent of the stratiform liquefactions and 45 per cent of the funnel liquefactions being manifest within four days, while only 6 per cent of the cup liquefactions were noted within the same period. Furthermore, 79 per cent of the cup liquefactions, 29 per cent of the funnel liquefactions, and 10 per cent of the stratiform liquefactions were not manifest until after the 10th day. We thus observe that a shortening of the time of incubation of gelatin cultures to 10 days or less, as has been the practice in some laboratories, would result in the exclusion of four-fifths of the cup liquefiers, and from one-tenth to one-fourth of the other forms from among the liquefying types, with a consequent increase in the reported presence of *B. coli* in samples from which it was undoubtedly absent. The records of the examination of over 23,000 cultures obtained in routine *B. coli* determinations during the past five years, amply substantiate the statement that a shortening of the gelatin incubation period would introduce a serious error into the analytical results.

An examination of the results obtained at 40° C. reveals the fact that 28 per cent of all the cultures tested produced liquefaction within 24 hours, and 34 per cent within four days, while 66 per cent of the cultures failed to produce any signs of liquefaction whatever during the period of four days that they were under observation. A certain similarity between the behavior of cultures of the cup, funnel, and stratiform types at 20° and at 40° C. is also apparent; 65 per cent of the cultures of the stratiform type and 46 per cent of those of the funnel type showed liquefaction at 40° C. within four days, while only 21 per cent of the slowly liquefying cup type were able to produce liquefaction at the higher temperature within the same period. About four-fifths of all cultures which were able to produce peptonization at all during four days at 40° C. did so within the first 24 hours. The rate of liquefaction at 20° C. and at 40° C. by cultures of these different types is shown in the following table.

TABLE 2.  
RELATIVE LIQUEFACTION AT 20° C. AND 40° C. BY CULTURES OF THE CUP, FUNNEL, AND STRATIFORM TYPES.

	All Cultures	Cup Type	Funnel Type	Stratiform Type
Number of cultures.....	288	153	106	29
Per cent. of cultures first showing liquefaction at 20° C. on the				
4th day	27	6	45	73
7th "	7	4	10	10
10th "	12	11	16	7
14th "	54	79	29	10
Per cent of cultures first showing liquefaction at 40° C. on the				
1st "	28	16	39	48
2d "	3	1	3	10
3d "	1	1	1	7
4th "	2	3	3	0
Per cent of cultures liquefying at 40° C.....	34	21	46	65
Per cent of cultures not liquefying at 40° C.....	66	79	54	35

There is apparently very little correlation between the rate of liquefaction at 20° C. and liquefaction at 40° C. To be sure, a much greater proportion of the cultures which had produced liquefaction at 20° C. within four days reacted within a like period at 40° C. than was the case with cultures which produced gelatinase more slowly at the lower temperature. For example, 74 per cent of the four-day 20° C. cultures liquefied within four days at 40° C., while only 11 per cent, 22 per cent, and 20 per cent, respectively, of the 7-, 10-, and 14-day cultures reacted within the same period. That is to say, about one-fifth of the cultures which were unable to produce lique-

faction within one week at 20° had their peptonizing function so accelerated by incubation at 40° that they were able to react within four days. On the other hand, about one-fourth of the cultures which liquefied within four days at 20° C. were unable to produce a like reaction in the same period at 40° C., although taking all the cultures into consideration there was an increase of about 6 per cent in liquefactions recorded within four days at 40° C. over those recorded in the same period at 20° C. The failure of so many cultures to produce peptonizing enzymes at 40° C. in the same time that they were able to do so at 20° C. is especially significant, as it indicates that the optimum temperature for enzyme production is quite different with different cultures, and further suggests that if this be the case, it is quite possible that by cultivation at the proper temperature, the liquefying function might be induced in many cultures which have hitherto been classed among the non-liquefying types. Jordan<sup>1</sup> has recently demonstrated that the optimum temperature for the reaction between the liquefying enzyme and the gelatin and the optimum temperature for the bacterial production of that enzyme are often widely different. The relative liquefaction at 40° C. by cultures which produced peptonization in 4, 7, 10, and 14 days, respectively, is shown in the following table.

TABLE 3.  
LIQUEFACTION AT 40° C. BY CULTURES LIQUEFYING IN 4, 7, 10, AND 14 DAYS RESPECTIVELY AT 20° C.

	Fourth Day	Seventh Day	Tenth Day	Fourteenth Day
Number of cultures.....	78	19	36	155
Per cent of cultures first showing lique- faction at 40° C. on the	<div> <div>1st day</div> <div>2d "</div> <div>3d "</div> <div>4th "</div> </div>	<div> <div>11</div> <div>0</div> <div>0</div> <div>0</div> </div>	<div> <div>18</div> <div>2</div> <div>2</div> <div>0</div> </div>	<div> <div>15</div> <div>1</div> <div>0</div> <div>4</div> </div>
Per cent of cultures liquefying at 40° C.....	74	11	22	20
Per cent of cultures not liquefying at 40° C.....	26	89	78	80

#### CONCLUSIONS.

The results of the foregoing experiments have been almost completely a record of failures, due undoubtedly to the fact that the fundamental assumption, that since the growth of bacteria of the types in question was more rapid at 40° C. than at 20° C., the produc-

<sup>1</sup> *Loc. cit.*

tion of liquefying enzymes must also be accelerated at the higher temperature, was untenable. We have learned by past experience that the other biochemical functions by whose aid we are able to separate from the colon group many types of foreign bacteria, the production of gas and acid in sugar solutions, the reduction of nitrates, the coagulation of milk, and the production of indol are more active at the higher temperature. We learn from these experiments, however, that the liquefying function does not as a rule follow the same law as the other biochemical functions.

Concerning the attempts to prepare a gelatin medium which should be solid at a temperature of 40° C. upon which liquefaction might be noted in the same manner as that employed with ordinary culture gelatin at 20° C., it need only be said that, while such media can be made, its preparation is difficult, and by the use of more recent methods of testing for liquefaction, its use becomes unnecessary in dealing with the specific problem—the shortening of the time involved in making tests for *B. coli*.

The use of broth cultures to accelerate the production of liquefying enzymes also appears to be of doubtful value. From the comparative experiments with gelatin cultures at 20° C. and at 40° C. we see that more than half of the liquefying cultures commonly found during routine *B. coli* tests at Lawrence were of one type, with about four-fifths of which liquefaction was not manifest until after the 10th day, and with this type of cultures liquefaction was not produced within four days, when they were incubated at 40° C. Furthermore, we note that a very considerable proportion of cultures of the funnel and stratiform types, which usually produce liquefaction within four to seven days at 20° C., failed to show liquefaction within four days at 40° C. That is to say, while the activity of the liquefying function was greatly increased at the higher temperature in some instances, it was in other cases materially retarded.

The temperature at which the liquefying enzymes act most energetically upon gelatin has been determined by a number of investigators. The determination of the temperature at which the greatest bacterial activity in enzyme production shall be combined with the maximum liquefying activity of that enzyme appears, however, to have been overlooked, and it appears to the writer that data of this



character are necessary before we may formulate a method for determining liquefaction which shall be at the same time rapid and accurate. The preparation of media more favorable for rapid enzyme production than are the usual culture media, and the establishment of more refined chemical tests for the detection of minute quantities of the liquefying enzymes would also appear to be fertile fields for investigation.

## THE USE OF LACTOSE-BILE MEDIUM IN WATER ANALYSIS.

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THE determination of intestinal bacteria as represented by *Bacillus coli* gives, when properly interpreted, a most valuable index of the quality of a water from a sanitary standpoint. Unfortunately the presumptive test with Smith solution, owing to the interference of other growths of bacteria has often proved to be misleading, especially in badly polluted waters.

In a paper previously published,<sup>1</sup> entitled "A New Solution for the Presumptive Test for *Bacillus coli*," the author has described numerous experiments on the selective inhibiting effect upon the growth of bacteria by the various salts and their constituents found in the bile. By the use of bile media most species of bacteria other than *B. coli* are killed or restrained, thus allowing of the free action of the intestinal germs. The tests show that the cholic acid radical of the bile is the effective agent. This conclusion is important in that it admits of the use of sodium glycocholate as an inhibiting salt. In ox bile, which is the material most available, nearly all of the bile salt present is in the form of glycocholate.

The experiments also show that good results are obtained only when an amount of bile salt is used equal to that present in undiluted ox bile but that further concentration is unnecessary. Liquid ox bile filtered after sterilization contains about 110 grams of solid matter per liter, of which about 90 grams consist of bile salts. This is an amount far in excess of that previously employed as a restraining agent but a lesser amount gives less efficient results. The liquid also contains albuminous matter equal in food value to the meat extract employed in the usual media.

An undiluted bile solution sterilized when freshly drawn may be

<sup>1</sup> *Biological Studies by the Pupils of William Thompson Sedgwick*, University of Chicago Press, 1906.

kept in stock until ready for use. It is then decanted or filtered and 1 per cent of lactose, previously dissolved in a small amount of water, is thoroughly mixed with the bile, after which it is tubed and sterilized in an autoclave for 30 minutes at 15 pounds pressure. The fermentation tubes employed are 140 mm. long and 15 mm. in diameter, having an elongated bulb 38 mm. in its shortest diameter. This admits of the addition of 10 c.c. of the water to be tested without too great dilution of the medium.

A large number of market samples of evaporated or inspissated bile were tested, but they were all found to be very acid, and even when neutralized did not give proper results. It has since been established by experiment that if the bile is sterilized in an autoclave when freshly drawn and the filtered liquid evaporated to dryness that this product when kept perfectly dry will remain practically neutral and can be preserved indefinitely. A combination of 110 grams of the solid bile and 10 grams of lactose dissolved in a liter of distilled water will give the same results as the medium made from fresh liquid bile.

It has been shown by extensive tables given in the article previously referred to that negative results for *B. coli* have been often found by the use of Smith solution when there was no question that the germ was present but had been overgrown in the medium and crowded out by other bacteria. On the other hand, experiments recently performed show that apparent positive tests for *B. coli* may be obtained when a mixture of gas producing bacilli other than *B. coli* are added to Smith solution.

The results so far obtained with bile lactose medium point strongly to the fact that no gas producer or mixture of gas producing bacteria will give results as high as 25 per cent of gas except *B. coli*, even when three days' incubation is employed. Attenuated *B. coli* sometimes requires three days for its development to that point. The necessity for the absorption test is therefore eliminated.

In testing a water for *B. coli* dilutions of 0.1, 1.0, and 10 c.c. are planted in the fermentation tubes and incubated at 37.5° C for 48 hours. All tubes giving over 25 per cent of gas are considered to contain *B. coli*. Those having any considerable amount of gas but still under 25 per cent are returned to the incubator for 24 hours longer.

Attenuated *B. coli*, as before stated, will sometimes require 72 hours to give the positive test.

Underground waters not exposed to surface influences and properly filtered waters should not give positive tests in any dilution. Surface springs, open dug wells, and pure surface waters may give positive tests in the 10 c.c. dilution, but in no less quantities. Moderately contaminated waters and those exposed to surface drainage containing small quantities of the excreta of the lower warm-blooded animals may give positive tests in the 1 and in the 10 c.c. dilutions. Such waters are to be looked upon with suspicion. Contaminated waters and those having a large amount of surface wash will give positive tests in the  $\frac{1}{10}$ , 1, and 10 c.c. dilutions. For grossly polluted waters and sewages higher dilutions may be employed, as in testing sewages and sewage effluents from filters.

At Mt. Prospect Laboratory about 5,000 samples of waters of various degrees of purity and from hundreds of different sources have been tested by means of lactose-bile media, and the results have always agreed with the judgments formed by the complete analysis of the water as well as by careful sanitary inspections of the individual sources of supply. In many instances contamination has been found to exist which would not otherwise have been detected.

The use of this medium gives an exact gauge of the purification brought about by systems of filtration, and in the New York City, Department of Water Supply, Gas and Electricity; six larges and filter beds and two mechanical filter plants, under the author's charge in the Borough of Brooklyn, are daily tested by this method as well as for quantitative bacterial removal.

CONCLUSIONS.—The bile medium previously employed has contained too little bile salt to be effective as a selective inhibiting agent.

The use of the lactose-bile medium herein described for the determination of *B. coli* prevents negative tests from the overgrowth of other bacteria as well as positive tests from mixtures of other gas formers.

By the employment of this new medium more definite results on the sanitary quality of a water may be obtained than by any other test at present in use. The medium is also especially valuable in the testing of filter plants.



## EXPERIENCE WITH LACTOSE-BILE MEDIUM FOR THE DETECTION OF *B. COLI* IN WATER.

LUTHER R. SAWIN.

(From Mt. Kisco Laboratory, Mt. Kisco, N. Y.)

THE discovery of *B. coli* in 1885 by Escherich, coupled with the knowledge that it is a normal inhabitant of the intestine of man and many animals, and therefore is found in sewage and polluted water, has induced water-bacteriologists to pay more attention to its isolation than to any other part of their work. Various methods have been proposed and tried for the detection of this organism in water. The one considered most reliable and meeting with general approval has involved the planting of a portion of the sample, either directly or after revivifying in broth, into plates of lactose-litmus agar; and then putting those colonies developing *B. coli* characteristics through the different tests as recommended for the identification of this organism. This procedure although accurate, is at the best a slow, tedious, and laborious process. Moreover, it is impracticable when used by the supervisor of a public water-supply, whose duty it is to report on the daily sanitary condition of the water. For example, if two days are required for the water to reach the inhabitants of the city, and it takes seven days to learn the results by this process, the consumer will have used the water five days before he is informed of the danger.

To obviate these difficulties, various rapid methods have been proposed and used by different water-analysts. Nearly all are based on the fact that *B. coli*, when grown in a fermentable sugar, generates a certain amount of gas of which a definite proportion is carbon dioxid. In many cases certain chemicals whose function it is to inhibit the growth of the water bacteria have been added to the sugar solution. The most common is phenol, either alone or in combination with hydrochloric acid. McConkey has recommended the addition of sodium taurocholate to agar; others have used this compound in Smith solution. It would seem that these different methods, all based on the same general principles, ought to give something like concordant results. But unfortunately this is not the case and they

do not check one another even within reasonable limits. The use of phenol has called forth considerable controversy as to its merits. In this laboratory, where it has been used more or less in an experimental way, it is the experience of the author, that when used to test sewage or badly polluted waters, it gives very satisfactory results, but when used to test a questionable water or one containing few *B. coli* or *B. coli* in an attenuated condition, it generally fails to give the positive test. In a case like this Smith solution alone is preferable. It is very evident that a method requiring a previous knowledge of the character of the water for its use is of very little value.

On the other hand, many bacteriologists, recognizing the uncertain value of phenol in media, prefer to use Smith solution without the addition of any reagent having an inhibitory effect. With the use of Smith solution the conditions are just reversed. And in this laboratory, where it has been used since its establishment in 1903, it has been found very satisfactory with good or questionable waters, but with sewage of badly polluted streams, it often completely fails. Frequently with sewages, the fermentation tubes show no gas production whatever, even when inoculated with very small quantities of the sewage.

As a solution of the problem attending the sanitary examination of water, Mr. Daniel D. Jackson,<sup>1</sup> of Mt. Prospect Laboratory, Brooklyn, N. Y., has proposed the use of lactose-bile medium for the fermentation test. This medium consists of undiluted liquid ox bile sterilized when freshly drawn and containing 10 grams of lactose to the liter of bile. The medium has been used in this laboratory for the last five months in conjunction with the presumptive test. In all 1,076 samples have been examined by both methods, and, as far as possible, waters of a varied character have been used ranging from sewages to waters whose purity is beyond question. Not only have comparisons been made as to the relative value of the two methods, but waters, whose sanitary condition is known by actual inspection of the local conditions have been examined with lactose-bile in view of ascertaining how far the results conform to the character of the water.

Aside from the fact that the presumptive test is unreliable in the examination of polluted waters, there is another factor which tends

<sup>1</sup> *Biological Studies by the Pupils of William Thompson Sedgwick*, University of Chicago Press, 1906.

to throw discredit on its results. Reference is now made to the large number of anomalies which this method furnishes. For example: if fermentation tubes of Smith solution are inoculated with 0.1, 1.0, and 10.0 c.c. portions of the sample, frequently a positive test is obtained in the 0.1 but not in the 1.0 and 10.0 c.c. tubes. And, what is still more puzzling, occasionally, the sample gives a test in the 0.1 and 10.0 c.c. inoculations, but not in the 1.0 c.c. With bile medium, while occasional anomalies have been found, there has been a great reduction, as may be seen from the following data: Of 1,063 samples, duplicate analyses were made in lactose-bile and Smith solution. In the 1.0 c.c. portions 2.73 per cent gave anomalies by the former and 8.00 per cent by the latter method. With the 10.0 c.c. portions, the bile medium gave 4.73 per cent against 13.73 per cent anomalies by Smith. In both cases, the reduction in anomalies was approximately two-thirds.

With the use of the presumptive test for *B. coli*, it has been the practice to consider the test positive, when the total gas production was 25-70 per cent of total volume, and the carbon dioxide 25-40 per cent of this amount. Whipple, Irons, and other authorities agree that the percentage of carbon dioxide should be about 33 per cent of total gas evolved. Experiments in this laboratory, with pure cultures of *B. coli* in fermentation tubes of Smith solution and lactose-bile show that with the former there is a wide range in the percentages of carbon dioxide found. Nineteen inoculations in Smith solution gave an average of 27 per cent carbon dioxide. With the lactose-bile, the results obtained were more uniform, and the average percentage of carbon dioxide obtained was appreciably higher, being 37 per cent. In actual experience, with tests on polluted streams, it has been found that the percentage of carbon dioxide as found by lactose-bile medium has been about 39 against 30 with Smith. Table 1 illustrates this point very clearly.

In this table, the 1-1,000 and 1-100 dilutions are samples of sewages and street wash from which *B. coli* have been repeatedly isolated. As other examples to illustrate the differences in the percentages of carbon dioxide as found by the two methods, tests were made on two badly polluted streams. The first receives sink drainage and street wash; the second, barnyard drainage and street wash. Twenty

TABLE 1.

DILUTION	SMITH SOLUTION		LACTOSE-BILE SOLUTION	
	No. Sample Examined	Per Cent CO <sub>2</sub>	No. Samples Examined	Per Cent CO <sub>2</sub>
1-1,000.....	9	30.00	14	39.17
1-100.....	14	27.40	26	39.62
1-10.....	352	30.51	214	39.93
1.....	526	29.29	430	39.65
10.....	649	29.71	618	38.89

samples from each stream were analyzed, and with the first, the average percentage of carbon dioxid (including 0.1, 1.0, and 10.0 c.c. portions) was 29.24 by Smith against 40.09 by lactose-bile. With the second stream, averages of 28.96 and 39.20 per cent carbon dioxid were found by tests with Smith and lactose-bile solutions, respectively.

In the second portion of this paper it is the object, first, to show the relative value of the two methods when used on different classes of waters; and second, and more important, to show how the results obtained by the lactose-bile method compare with the known facts ascertained by inspection of the local conditions in which the waters under consideration are found.

For this purpose, series representing polluted and unpolluted waters have been selected and arranged according to their sanitary qualities as shown in Table 2, and the average percentage of samples giving positive tests for *B. coli* with Smith and lactose-bile solutions recorded in the subsequent columns. For the wells, these figures are an average of 50 analyses; for the surface waters and sewage, an average of about 20 analyses. Samples numbered 1 are waters from deep, driven wells not open at the top; those numbered 2 are waters from shallow, open, dug wells. Waters numbered 3, 4, 5, 6, 7, and 8 are from lakes situated in sparsely inhabited regions where there is little danger of pollution. The next group of waters (Nos. 9, 10, and 11) are more or less polluted. No. 12 is a badly polluted stream; No. 13 principally a mixture of street wash and household drainage; No. 14 is a septic sewage.

An examination of Table 2 shows that, with the first eight waters, there is very little difference in the figures obtained by either method. With well waters, it is extremely rare that *B. coli* is found in



the 10 c.c., and it has never been found in the 0.1 and 1.0 c.c. portions although large numbers of these waters have been examined with lactose-bile medium. With the waters from the lakes, which are situated in sparsely inhabited regions, the small amounts of coli are due, no doubt, to the effect of small animals. The results in all these samples indicate waters of an excellent quality.

With the next three waters, which are more or less polluted, the percentages of positive tests with the bile medium are appreciably higher than those found with the Smith solution. The results with the lactose-bile would seem to be more in accordance with the facts. With this group of waters, at times *B. coli* can be found and at others it cannot be detected with Wurtz agar.

If, from the preceeding results, there is any question as to the relative value of the two methods, or as to the value of the lactose-bile method when used for the detection of *B. coli* in polluted waters, it is removed after examining the results which samples Nos. 12, 13, and 14 furnish. In every case analyses by the lactose-bile method gave decidedly higher results than by the Smith; and in the 1.0 and 10.0 c.c. portions the percentages of positive tests were close to 100. With the sewage, while there was an occasional negative result, about 90 per cent of the tests were positive with the bile against only 25 per cent with the Smith. With the three samples, now under

TABLE 2.

No.	SOURCE	PERCENTAGE OF SAMPLES GIVING POSITIVE SMITH SOLUTION			TESTS FOR <i>B. coli</i> BY LAC- TOSE-BILE MEDIUM		
		0.1 C.C.	1.0 C.C.	10.0 C.C.	0.1 C.C.	1.0 C.C.	10.0 C.C.
1	Deep wells.....	0.	0.	0.	0.	0.	0.
2	Shallow wells.....	0.	1.0	10.0	0.	0.	6.0
3	Lake.....	15.0	10.0	15.0	5.0	0.	15.0
4	Lake.....	5.2	15.7	21.0	5.2	5.2	31.0
5	Lake.....	10.0	5.0	40.0	10.0	5.0	15.0
6	Lake.....	10.0	10.5	26.0	10.0	10.5	50.0
7	Lake.....	0.	10.0	5.0	00.	0.0	15.0
8	Lake.....	10.0	15.0	35.0	0.	5.0	30.0
	Average, Nos. 3, 4, 5, 6, 7, and 8....	8.3	11.0	23.6	5.0	4.3	26.0
9	River.....	47.0	72.2	55.5	50.0	75.0	84.2
10	River.....	26.3	37.6	73.7	30.0	90.0	85.0
11	River.....	36.8	55.1	68.0	40.0	73.5	78.1
	Average, Nos. 9, 10, 11.....	36.7	55.1	66.0	40.0	79.4	82.4
12	Brook.....	47.7	63.2	72.2	60.0	90.0	84.2
13	Drainage.....	50.0	73.7	78.9	84.2	90.0	90.0
14	Sewage.....	25.0	25.0	8.2	87.5	93.7	81.2
	Average, Nos. 12, 13, 14.....	40.9	53.9	53.1	77.2	91.2	85.1

consideration, the averages of the positive tests obtained by the two methods show that the bile gave nearly twice as many positive tests as the Smith. These three samples give results corresponding very closely to what one would expect and what one should find in the examination of polluted waters.

#### SUMMARY.

The presumptive test, using lactose-bile medium, is a rapid and practical method for the detection of *B. coli*. If the absorptions are omitted, as the data obtained from experiments seem to show that they may be without detracting from the value of the test, the routine work is generally lessened.

The anomalies obtained by this method are few and much less than when Smith solution is employed.

The percentage of carbon dioxide is decidedly higher when lactose-bile is used for testing waters than when Smith solution is employed. This fact corresponds with the results obtained by experiments with pure cultures of *B. coli* in lactose-bile and Smith solutions.

Both methods give substantially the same results when used for testing "safe" waters, and the results are consistent with the facts learned by observation. With questionable or contaminated waters, the percentage of positive tests obtained with the use of lactose-bile media are appreciably higher than those obtained with the use of Smith solution. With sewages or badly polluted waters, lactose-bile medium gives a decidedly greater number of positive tests than the Smith solution.

For general convenience in the routine work of a laboratory, for practicability in the examination of public water-supplies, and for reliability in testing waters of a varied character, the fermentation test, using lactose-bile medium, is the most promising method that has yet been advanced.

# COMPARATIVE RESULTS OBTAINED BY THE USE OF LACTOSE-BILE AND DEXTROSE-BROTH MEDIA FOR THE DETECTION OF *B. COLI* IN WATER.

ROBERT SPURR WESTON AND RALPH E. TARBETT

FOR some little time past comparative tests have been made with the use of dextrose-broth and lactose-bile media in the preliminary enrichment tubes in the examination for *B. coli* in the raw and filtered water of the Tennessee River at the Laboratory of the Knoxville Water Company, Knoxville, Tennessee.

The lactose bile has been prepared from fresh local ox-bile to which 1 per cent of lactose has been added just before sterilization. The dextrose-broth has been prepared according to the directions given in the "Standard Methods of Water Analysis."<sup>1</sup>

The method used in the isolation of *B. coli* has been that prescribed by the Standard Methods. We have not, however, attempted to rejuvenate, by successive enrichment, debilitated forms which have lost some of their powers to react with the various media.

In recording results failure to give the correct gas ratio with dextrose-broth, or failure to give the indol reaction with the peptone solution, have been classed as atypical, while cultures which fail owing to the liquefaction of gelatin have been classed as belonging to the *B. cloacae* group. We have made comparative results with 63 samples of water. Of this number, 21 gave no gas formation with either media, 18 gave gas formation with both media, and 24 gave gas formation with dextrose-broth, but not with lactose-bile.

It has been our observation that the formation of gas takes place much less rapidly in the lactose-bile than in the dextrose-broth medium, there being a comparatively small amount of gas formed in the first 24 hours. In some cases more than 24 hours were required before the appearance of gas was noted in the lactose-bile solution. In no case did the amount of gas formed in 48 hours exceed 65 per cent of the closed arm of the tube, while the average amount was

<sup>1</sup> *Jour. Infect. Dis.*, Suppl. 1, 1905, p. 1.

43 per cent. The ratio of  $\text{CO}_2$  to the total gas in the tubes containing lactose bile varied very much as the ratio varies in the case of dextrose broth.

The isolation results obtained from the preliminary cultivation tubes of the 18 samples which gave gas formation with both dextrose-broth and lactose-bile are as follows:

No. Samples	Dextrose-Broth	Lactose-Bile
4	Typical <i>B. coli</i>	Typical <i>B. coli</i>
1	Typical <i>B. coli</i>	Atypical form
1	Streptococci	Typical <i>B. coli</i>
3	Atypical forms	Atypical forms
1	Streptococci	Atypical form
4	<i>B. cloacae</i>	Atypical forms
4	<i>B. cloacae</i>	<i>B. cloacae</i>

It is seen that the results of the isolation from the lactose-bile enrichment were with four exceptions either the typical *B. coli* or very closely related forms. The microscopical examination of the cultures from the lactose-bile enrichment tubes showed pure cultures whose physical characteristics agreed with those of *B. coli*.

It is possible that, in those cases where atypical results were obtained, positive results might have been obtained by further rejuvenation in nutrient broth.

Of the 24 samples showing gas formation with dextrose-broth but not with lactose-bile, the following results were obtained:

No. Samples	Dextrose-Broth
1	. . Typical <i>B. coli</i>
7	. . Atypical forms
2	. . Streptococci
2	. . <i>B. cloacae</i>
9	. . Failure on litmus lactose agar
3	. . Negative forms not classified above

We make no comments on the results, but simply state the facts as observed thus far by us in the comparative use of the two media.



## SANITARY CHEMICAL EXAMINATION OF WATER BACTERIA.<sup>1</sup>

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IN view of the modern theory of disease the problem of sanitary water analysis concentrates itself on the bacteriological rather than on the chemical condition of the water in question, whatever the method may be of arriving at this knowledge. All waters would be perfectly safe for drinking purposes, with certain well-characterized exceptions in the case of mineral constituents, if only the pathogenic bacteria were removed. Our investigations are, therefore, directed toward those bacteria that are pathogenic to man, are capable of being borne by water, and have the possibility of infecting the human subject through the alimentary tract.

The routine sanitary chemical tests, all of which are for substances perfectly harmless in themselves, are merely an indirect method of determining the bacteriological condition of the water. Naturally with such an indirect method there is a very wide range of possibilities in the interpretation of the results. The analytical data of a sanitary chemical analysis indicate merely the presence, recent presence, or possible future presence of pathogenic bacteria. It follows accordingly that by current methods the sanitary chemical analysis of pure distilled water to which a little sterile bouillon had been added would indicate a dangerously polluted water.

Naturally, with the advancement of bacteriology, more direct methods of analysis have been undertaken. These are of course limited to quantitative and qualitative investigations of the bacteria. One does not need to emphasize the difficulty of establishing a standard for the maximum number of bacteria permissible in a potable water, or of the uselessness of searching the Mississippi River or the Great Lakes for a typhoid bacillus.

<sup>1</sup> This work was carried on under co-operative agreement between the Illinois State Water Survey, State Geological Survey, Engineering Experiment Station of the University of Illinois, and the Division of Hydro-Economics of the United States Geological Survey.

The only qualitative work attempted at present is the identification of intestinal forms. The presumptive coli tests are in a very uncertain and unsatisfactory condition. The quantitative estimation of coli is well-nigh impossible in a laboratory where a large number of samples are received for daily routine examination. Though possible presence or absence can be quite satisfactorily made out, even when many samples require attention at the same time, an opinion cannot be based on a qualitative test alone. It is essential to know the quantity of coli bacteria present.

Of the various routine methods of procedure, the direct chemical analysis, being the broadest of all, has one advantage over all, or one disadvantage as the case may be. Such an analysis does not depend on the presence of living active organisms as do all bacterial methods; but the pollution may still be detected where bacteria have died in large quantities after exhausting their food material. Also the chemical analysis will detect the pollution where soil-filtered sewage reaches a water-supply. Probably the most necessary factor in interpreting the sanitary chemical analysis is a thorough knowledge of the source of the water under examination. In this section of Illinois one is utterly helpless in the interpretation of the analysis of a well water, unless he knows whether the well is deep or shallow and in drift or in rock.

The results obtained by analyzing the water from two wells in Urbana will illustrate the difficulty. Amounts are stated in parts per million.

	No. 1	No. 2
Total residue on evaporation . . . . .	415.	365.
Chlorine in chlorides . . . . .	3.5	7.5
Oxygen consumed . . . . .	5.2	2.0
Nitrogen as free ammonia . . . . .	3.6	0.054
Nitrogen as albuminoid ammonia . . . . .	0.136	0.056
Nitrogen as nitrites . . . . .	0.040	0.030
Alkalinity . . . . .	353.	245.
	1 c.c.	1 c.c.
Bacteria at 20° C. . . . .	200.	1000.
<i>B. coli communis</i> . . . . .	Absent	Present

No. 1 is from a well 180 feet deep and has high oxygen consumed and high free and albuminoid ammonia, which are characteristic of deep wells in the drift in central Illinois. Bacterial tests show that the water is in good condition.

No. 2 is a water from a 30-foot dug well, and chemically, according to usual methods of interpretation, it is a better water than No. 1. Bacterially it contains 1,000 bacteria per c.c., and the reaction is positive to the presumptive test for coli.

Generally speaking, the chemist must know the normal constituents of a given locality before he can determine the amount due to pollution.

In addition to the sanitary chemical analysis and the quantitative and qualitative bacteriological examination, a further line of procedure is suggested to the scientist, that is, the chemical analysis of bacterial cultures obtained by the inoculation of water samples into artificial media.

Our experiments upon this principle are based on the supposition that, by the inoculation of water into artificial sterile media, we could, in a way, imitate the changes that would ordinarily be brought about by bacteria in a water containing natural media. One great advantage would lie in the ease with which the artificial media could be analyzed, and the accuracy with which the changes due to the water could be determined. By this means it is possible that local factors which affect the interpretation of the results of analysis may be removed. There arises the possibility of establishing an absolute standard for the maximum limits of impurities.

To establish the value from the sanitary standpoint of the analysis of the cultures of water, our experiments are carried out with a view to securing constancy of results. After a few preliminary tests (see Table 1) we chose for a medium an ordinary meat-extract broth of double concentration, to which 2 per cent of gelatin was added. The presence of sugars prevented decomposition of the nitrogenous constituents. When it was desired to study the nitrogenous decomposition products, sugars were not added to the medium, although the extremely small amount of sugar present in ordinary acid meat extract was not removed.

For our first experiments we chose two types of water, one from a small creek, known to be seriously polluted, the other from deep, driven wells of unquestionable purity. We measured accurately 5 c.c. of each medium into test-tubes, inoculated with 1 c.c. of each sample of water, and incubated at 37° C. In these preliminary tests, the cultures, when they showed appreciable differences, were steamed in an Arnold sterilizer 20 minutes, diluted to 1,000 c.c., and subjected to sanitary analysis. *A priori* it was expected that it would be necessary to work with very young cultures, presuming that for old cultures the ultimate analysis of a pure and of a polluted water would be very similar. The waters tested thus far have given decidedly the contrary result. Table 1 is characteristic of the effect of

variations in media and age of culture. The results are expressed in parts per million of the diluted culture, 5 c.c. of the inoculated medium diluted to 1,000 c.c. with pure distilled water.

TABLE 1.  
COMPARISONS OF THE ACTION OF PURE AND POLLUTED WATER ON MEDIA OF VARIOUS COMPOSITIONS FOR DIFFERENT PERIODS OF TIME.

SAMPLE	OXYGEN CONSUMED		Acidity		AMMONIA				TOTAL SOLIDS		NITRATES		CHLO- RIDES	MEDIA
					Free		Albuminoid							
Hours...	24 hrs.	89 hrs.	24 hrs.	89 hrs.	24 hrs.	89 hrs.	24 hrs.	89 hrs.	24 hrs.	89 hrs.	24 hrs.	89 hrs.	89 hrs.	Broth
Tap.....	102	75	12.13	12.13	0.64	1.12	8.00	13.6	... 212	0.48	0.40	37.0		
Creek.....	90	55	12.13	21.82	4.60	13.20	8.00	4.8	... 124	0.40	0.44	35.5		
Control...		75		7.27		0.64		6.80		254	0.48	37.5		
Tap.....	165	170	16.97	15.52	0.72	1.00	8.80	14.	... 386	.... 0.40		36.0	Glucose Broth	
Creek.....	145	145	36.37	54.80	0.76	1.00	7.60	12.0	... 309	.... 0.72	36.0			
Control..		165		14.55		0.64		6.4		433	0.52	35.0		
Tap.....	94	92	14.55	19.40	0.52	1.04	18.80	23.2	428 308	.... .56		34.5		
Creek.....	104	60	24.25	67.90	3.60	23.20	18.80	10.4	397 168	.... 0.64		32.5	Gelatin Broth	
Control..		85		9.70		0.64		13.2		359	0.52	32.5		
Tap.....	185	183	21.82	26.67	0.76	1.12	18.00	23.20	612 489	0.72 0.48		36.5	Gelatin Glucose Broth	
Creek.....	195	163	33.95	60.62	0.84	1.36	19.20	22.4	574 400	0.80 0.60		36.0		
Control..		180		16.97		0.64		13.60		557	0.44	37.0		

As the most striking changes took place in the nitrogenous constituents, we decided to secure a series of preliminary tests determining only free ammonia. We were sometimes able to nesslerize directly, though more frequently, on direct nesslerization, we secured a greenish color, not comparable with a true nessler color.

In an attempt to establish a maximum limit of free ammonia, allowable, we obtained samples from deep wells of unquestionable purity, from the best available shallow wells, and from chemically pure water artificially polluted. In the following table the cultures were analyzed at different ages as a sufficient variety of waters had not yet been analyzed to determine the most favorable age of culture.

In Table 2 the following facts are of special interest. The previous statement that the chemical analysis of pure, deep, and shallow ground waters is entirely different is verified. Noticeable examples are Nos. 18 and 19. In No. 18 and No. 19 the results of the bacteriological analysis are practically the same and also the chemical analysis of the cultures of the two agree very well. This strengthens our hope that a universal standard of purity may be established.



TABLE 2.  
COMPARISON OF SANITARY ANALYSES OF WATERS, FROM VARIOUS SOURCES, WITH THE AMMONIA THAT IS OBTAINED FROM MEDIA BY INOCULATION

SAMPLE		BACTERIAL ANALYSIS		CHEMICAL ANALYSIS, PARTS PER MILLION							ANALYSIS OF CULTURE, P. M. 1-200 DIL.				
No.	Source	Lab. No.	No. per c.c.	Coli in one c.c.	Nitrogen as				Total Solids	Oxygen Consumed	Alkalinity	Chlorides	Ammonia		Age Cult. in hrs.
					Free	Albuminoid	Nitrites	Nitrates					Free	Alb.	
1	Tap, deep, driven wells.....		200	—	3.6	0.136	0.004	0.04	415	5.2	353	3.5	0.36	....	24
2	"Boneyard" polluted creek.....		17,000	+	2.8	0.64	0.015	0.585	325	10.75	374.4	45.0	5.86	....	24
3	Control.....	Same as No. 1 (above)											1.00	....	..
4	Tap, deep, driven wells.....	Same as No. 1 (above)											0.12	....	16
5	Driven wells.....	Same as No. 2 (above)											0.56	12.0	60
6	"Boneyard".....	Same as No. 2 (above)											1.52	....	16
7	Control.....	Same as No. 2 (above)											18.00	5.2	60
8	Vermillion River, raw.....	14725	2,600	+	0.120	0.336	0.000	0.40	350	7.15	234.7	3.5	1.20	....	68
9	Vermillion River, filtered.....	14734	120	7+	0.024	0.144	0.01	0.32	322	4.05	215.3	4.0	1.76	....	68
10	Control.....	14827	14,701	+	0.020	0.004	0.004	0.32	608	.....	302.6	16.0	0.94	....	63 1/2
11	Country well, 30 feet deep.....	Same as No. 1 (above)											0.2	....	63 1/2
12	Tap, deep, driven wells.....		700	+	0.014	0.048	0.012	0.548	572	2.25	253.4	22.5	1.4	....	48
13	Shallow well, 30 feet.....	14978	1,000	+	0.051	0.06	0.030	0.190	365	2.95	245.7	7.5	1.4	....	48
14	Deep well, driven.....	14980	120	—	1.768	0.06	0.000	0.36	384	2.75	353.2	7.5	0.16	....	48
15	Shallow well, 30 feet.....	14981	120	—	0.40	0.128	0.125	7.08	384	1.00	334.2	21.5	0.16	....	48
16	Shallow well, 40 feet.....	14984	10	—	0.82	0.04	0.000	0.610	520	1.50	310.8	14.0	0.08	....	48
17	Driven well.....	14986	19	—	0.88	0.06	0.000	0.028	475	1.7	291	14.0	0.08	....	48
18	Driven well, 30 feet.....	14987	33	—	0.88	0.082	0.000	0.40	501	2.75	251	13.5	0.20	....	48
19	Driven well, 15 feet.....	14988	10	—	3.60	0.164	0.000	0.04	439	6.4	350	3.5	0.00	....	48
20	Shallow well, 15 feet.....	14989	55	—	0.93	0.004	0.000	0.32	342	1.65	301	6.5	0.08	....	48
21	"Boneyard", polluted creek.....	14990	62	—	2.8	0.04	0.015	0.565	325	10.75	374.4	45.0	9.40	....	48
22	Maximum for this locality for shallow well.....	14992	55,000	+	0.02	0.05	0.001	5.0	500	2.0	.....	15.0	1.72	....	..
23	Sewage 1-1,000 dilution.....		500	+	0.032	0.016	0.000	0.010	.....	0.6	.....	..	1.72	....	48
24	Sewage 1-10,000 dilution.....		150	—	0.018	0.010	0.000	0.00	.....	0.3	.....	..	0.20	....	48

Analyses No. 7 and No. 8, raw and filtered river waters, furnish another good example to support our theory. On the whole, direct chemical analyses do not show the true efficiency of a water-filtration plant, as the results approximate each other rather closely. The bacteriological analyses show decided differences, about 97 per cent of the bacteria having been removed by filtration. The chemical condition of the two cultures varies widely. Ordinarily this would be adding comparatively little to the results of colony counts which show the bacterial efficiency of the filter; but in this case, although the efficiency of the filter was 95.4 per cent (there being only 120 bacteria per c.c. in the filtered product) the water responded to presumptive coli tests. After a long series of cultures no typical coli communis could be isolated, while the culture analysis was obtained on the third day with comparatively little effort. Even this time was unnecessarily long. It seems to us that the relation of this test to the presumptive coli tests will bear further investigation.

The analysis of cultures may also show qualitative as well as quantitative differences in water as indicated in samples No. 9 and No. 10. Here the chemical analyses are comparable and the number of bacteria practically the same. There is a difference in the quality of the bacteria as shown by the presumptive coli test and this difference shows up distinctly upon analyzing the culture. No. 9, which gave the positive coli test, gave 4.8 parts of free ammonia to the culture, while No. 10, which gave the negative test for coli, gave only 0.2 parts of free ammonia to the culture.

Realizing the importance of nitrite determinations in water analysis, we undertook to study their action. As indicated in our first table, the action of nitrites and nitrates could best be studied in media to which nitrites and nitrates had been added. We accordingly added 0.05 per cent sodium nitrite ( $\text{NaNO}_2$ ) to part of our ordinary media. The results of a single experiment were as follows:

#### NITRITE MEDIA CULTURES.

Tap water gave . . . . .	7.5	parts	per	million	N	as	nitrite.
Boneyard gave . . . . .	0.0	"	"	"	"	"	"
Media Control gave . . . . .	7.5	"	"	"	"	"	"

The above test, we believe, differentiated very sharply in this instance, between the nitrites formed by putrefactive bacteria and the nitrites normally very high in a pure water.

According to present methods of interpretation a good water may be condemned on high nitrites alone; but the absence of nitrites does not show that a water is good. Further, the presence of nitrites in shallow wells is assumed to be due not to normal constituents of the soil, but to bacterial action. It is still further assumed that the bacteria concerned are putrefactive forms. Possibly the extent of the assumption is not thoroughly understood. Deep ground waters often contain high nitrites, their presence being explained by the supposition of the reduction of the nitrates present by the ferrous iron. We have, however, isolated very active denitrifying bacteria from these waters, and also from the air. Such bacteria might very naturally gain access to shallow wells containing nitrates and form nitrites. As compared with the relatively few denitrifying bacteria, there is the great mass of nitrifying bacteria universally present in the soil and upon which the preservation of life upon the face of the earth depends. When we consider these two great classes of bacteria it does not seem to us to be proven that the presence of nitrites should condemn a surface water.

The sanitary chemical tests, developed as they were on an entirely empirical basis, before the science of bacteriology was scarcely begun, were naturally difficult of interpretation and mistakes were unavoidable.<sup>1</sup> Our experiments indicate that the present interpretation of nitrites is partly in error, in that high nitrites could not normally be present in a shallow well.

Except in extreme cases it is often impossible to give an opinion of an Illinois water from the ordinary sanitary data, chemical and bacteriological, simply on account of the lack of evidence furnished concerning the source of the sample. Other authors have had the same experience. M. O. Leighton<sup>2</sup> says:

There has been in the past surprisingly little discrimination used with reference to the selection of determinations for specific purposes. . . . If . . . it is desired to determine the amount of organic pollution in a water and show its value for domestic use, the chemist forthwith begins his round of nitrogen determinations, and closes with a statement of the oxygen consumed and the number of bacteria per c.c. In only a few well-known laboratories has this rule been violated, and such is the conservatism in the chemical profession that it will probably be largely followed in

<sup>1</sup> W. P. Mason, *Examination of Water*, 2d ed., New York; criticism of Wanklyn.

<sup>2</sup> T. M. Prudden, *Drinking-Water and Ice Supplies*, 2d ed., New York, 1901.

*Field Assay of Water*, Water Supply and Irrigation Paper, No. 151, pp. 10 and 11.

the future. . . . The occasional isolated sanitary analysis of a water is positively without value. There are throughout the country numerous state, municipal, and private laboratories in which sanitary analyses are carried on. The water analyzed may be today from a well, tomorrow from a brook, and the next day from a pond. From the results of a single analysis wise and ponderous verdicts are sent broadcast, and the eager, waiting public is duly impressed.

As the basis of our experiments we have taken up the interpretation of chemical data from a theoretical standpoint, and we believe that this standpoint is absolutely essential to the intelligent understanding of sanitary data. For example, oxygen consumed, nitrites, nitrates, total solids, albuminoid ammonia, and chlorides, when *high*, indicate putrefactive bacteria. Putrefactive bacteria under proper conditions *reduce*, often very strikingly, the oxygen consumed, nitrites, nitrates, total solids, and albuminoid ammonia. These statements are in perfect harmony. (Septic tank data would confirm this.) Thus as the organic matter is converted to inorganic matter, the oxygen consumed (organic) decreases as the free ammonia (inorganic) increases.

The culture tests, since they discriminate sharply between putrefactive changes and normal variations, seem especially adapted for water analysis. The specific tests for sanitary purposes, which seem to be especially applicable, are free ammonia and nitrites.

In its present stage of development we recognize that, as in all other methods, no distinction is made between pollution from animal and human sewage. At times this distinction may be of paramount importance when we consider that typhoid bacteria are neither harbored by lower animals nor multiply in natural water.

We realize that the foregoing experiments can be regarded only as preliminary. Before absolute conclusions can be drawn more experimental work must be done. We would suggest the following topics:

Experiments to illustrate the action of pure cultures of water-borne bacteria on media of various composition.

Experiments to determine the proportion of cultural changes due to the bacteria and to the chemical condition of the inoculated water samples.

Experiments with media of different composition, with especial reference to acidity, and nitrogen, sulphur, and carbohydrate compounds.

Study of both the (1) formation of putrefactive products and (2) their fate when added to the original media; e. g., the formation of nitrites from nitrite-free media rich in nitrates or saline ammonia and their removal when added directly to the original



media, or the formation of free ammonia from proteids or its reactions when added to the original media.

Further comparisons of water known to be pure and of water known to be polluted, and artificial pollutions with sewages and pure cultures, guarding against any errors that might arise from overgrowths.

Experiments with cultures of different ages and temperatures.

Experiments on the possibility of mailing small un-iced samples. The forms ordinarily multiplying under these conditions are not the ones which develop at 37°.<sup>1</sup>

Put pure cultures in water, dilute and incubate with different dilutions.

It is our hope that other investigators in the field of water analysis may take up with us the study along the lines suggested. In closely related work on the bio-chemistry of sewage filters Gage and Clark<sup>2</sup> have already reported considerable success.

In conclusion we wish to express our thanks to Dr. Edward Bartow for his invaluable co-operation in the development of the theoretical and practical considerations involved in the work and to Mr. P. C. Jeans and Mr. J. M. Lindgren who have rendered material assistance in carrying on the analytical work.

<sup>1</sup> Prescott and Winslow, *Elements of Water Bacteria*, New York, 1904.

<sup>2</sup> *Eng. News*, 1905, 53, p. 27, and *Jour. Am. Chem. Soc.*, 1905, 27, p. 327.

## THE RELATIVE IMPORTANCE OF STREPTOCOCCI AND LEUCOCYTES IN MILK.

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IN the general crusade for the public weal that has been carried on in this country for the last decade, much has been accomplished in providing for a more wholesome milk-supply in several of the great centers of population. Of especial interest in this connection are the records of the Department of Health of the City of New York<sup>1</sup> which show what has been accomplished in that place, and demonstrate what can be done elsewhere if apathy, ignorance, and civic wrongdoing could be overcome.

Notwithstanding advances, there yet remain problems to be solved and differences of opinion to be adjusted before it can be said that our ideas of fitting sanitary standards have been crystallized or brought into unison.

Probably two of the most important questions at present under discussion are those concerned with the presence and significance of streptococci and of "pus cells" in milk-supplies. Thus, according to the standards raised by one authority or another, a milk is adjudged good or bad. In this confusion of what should be considered a proper standard lie the difficulties and differences of opinion. To the writer it seems that the time is now ripe for a full and free discussion of the subject, and that a careful inquiry should be instituted to consider the problem in all its phases and to advise regarding the establishment of suitable standards.

The scope of this paper will be limited to a brief presentation and discussion of these topics, with criticisms and suggestions.

It is now generally agreed that it is a rare thing to be able to draw sterile milk in any moderate quantity from the udders of cows, even while using the greatest of precautions against contamination from the outside; witness the researches of Ward,<sup>2</sup> Reed and Ward,<sup>3</sup> Boekhout and de Vries,<sup>4</sup> von Freudenreich,<sup>5</sup> Conn,<sup>6</sup> Harrison and Cumming,<sup>7</sup> and many others. Such being the case, it is important

to know of what species are those bacteria which inhabit the milk ducts and teat-canals.

These may be divided into two main groups; first, those producing lactic acid fermentation, and second, those producing none. Of the former there exist two types, one giving rise during the fermentation to gas, commonly spoken of as the "*B. aerogenes* type;" the other giving rise to no gas and spoken of as the "*B. lactis acidii* type." Of the latter, i. e., the non-acid group, there exists a variety of forms that are apparently concerned in the causation of odors, flavors, etc., and which at times may even give rise to putrefactive changes. By far the most common, however, of all those bacteria are the lactic acid forms which in market milk, according to many authorities, run from 50 to 100 per cent, and in milk drawn from the udders, according to Harrison and Cumming, 95 per cent.<sup>7</sup>

Concerning the constitution of these acid-forming types, it is generally agreed that to the "aerogenes" type belong *B. aerogenes* (Escherich) for the most part, and in less degree *B. coli*; but all investigators are not of one mind concerning the identity of the bacteria entering into the type "*B. lactis acidii*" (Leichmann).<sup>8</sup> The literature, for example, contains descriptions of organisms under such names as *B. acidii lactici*, *B. lactis acidii*, *Mic. acidii lactici*, *Streptococcus acidii lactici*, *Streptococcus lacticus*, etc., all concerned in the production of lactic acid and coagulation of casein.

A careful consideration of the descriptions of these several bacteria, as given in the literature, leads one to believe that the points of difference lie largely in relatively insignificant variations induced by the non-uniformity of media, or of technique, or in questions of interpretation of results.

The question of the probable unity of the varieties described was first prominently brought forward by Kruse<sup>9</sup> of Bonn in 1903, who seriously doubted the bacillary nature of the lactic acid bacteria of the "*B. lactis acidii*" type, declaring the same to be streptococci closely related on the one hand to *Strept. pyogenes*, and on the other to *Mic. lanceolatus*. His paper, however, was not accompanied by any experimental data, although it must be inferred that he had conducted experiments leading him to this belief. He assigned a secondary rôle to *B. aerogenes* and *B. coli* as lactic acid formers in naturally soured milk.

In the following year there appeared from his laboratory a contribution on this subject by Hölling,<sup>10</sup> containing much experimental evidence of a convincing nature, wherein it is shown that streptococci are in reality the ordinary and most frequent lactic acid formers, and that *B. lactis acidi* and others of that ilk are not bacilli, but streptococci. With the exception of *B. aerogenes* and *B. coli*, this statement covers all previously described organisms recognized by various authorities as being those concerned in the primary lactic acid fermentation of fresh milk.

In ignorance of the papers of Kruse and Hölling, Heinemann,<sup>11</sup> working in the University of Chicago, in 1905, carried out examinations upon milk obtained from the udders of 42 cows under the best of precautions against contamination from the outside, and likewise upon samples of market milk in Chicago, with the object of ascertaining the nature of the bacteria concerned in the souring of milk, together with an inquiry into the status of "*B. lactis acidi*." For this latter part of the work pure cultures of this bacterium were obtained from several laboratories in this country, and also a series of "starters" such as are sold to dairymen for the ripening of cream. In all these investigations he found that there was but one organism concerned, and that organism was not a bacillus but a streptococcus, and, that in milk naturally soured, forms corresponding to *B. aerogenes* and *B. coli* were numerically less frequent. In this work he confirmed the remarks of Kruse<sup>9</sup> and the experimental data and conclusions of Hölling.<sup>10</sup> Further, he succeeded in isolating from separator slime, cows' feces, and in washing from the cows' udders and teats lactic acid bacteria corresponding in all particulars with the foregoing streptococcus type.

It is not to be inferred that, previous to these investigations, observers had not recorded the occurrence of streptococci in milk. The literature, which need not here be quoted, teems with records of such. But what seems strange is the fact that none regarded his results as other than an expression of a pathological state of the cow's udder, whilst "*B. lactis acidi*," regarded as a normal inhabitant, was passed by unnoticed as being of the same species, largely it would seem upon the ground of morphology, the bacterium occurring in pairs or short chains of slightly elongated individuals, an expression



of active binary division often seen in cultures of pathogenic streptococci.

In so far as morphological and cultural characters were concerned, Heinemann<sup>11</sup> found, on comparing a series of streptococci from pathological sources, milk, pure cultures of "*B. lactis acidi*," "starters," feces, etc., that there was perfect accord; in all, to be sure, there occurred from time to time variations of a parallel nature in both conditions.

Now, if we regard "*B. lactis acidi*" and *Strept. lacticus*, as Kruse and Hölling call it, to be identical (as I believe they are), in what light, then, are we to regard the presence of streptococci in milk as being an index of disease in the cow? It cannot be denied that cows suffer from inflammation of the udder at one time or another during lactation, and that these lesions are largely caused by the ordinary pyogenic cocci, less frequently by *B. coli* or *B. aerogenes*. Of the cocci it would seem that a streptococcus is the more prominent factor, judging from the researches of Steiger,<sup>12</sup> Bergey,<sup>13</sup> and others, particularly in that form of mastitis known as acute contagious mastitis.

But were we able to distinguish safely the several races of streptococci, it would then be a relatively simple matter to frame standards whereby we might be able to say that a given sample of milk was fit for consumption, whilst another was not, on the grounds that each contained harmless and hurtful cocci, respectively. In this matter we are yet without a test that will permit of assuming a dictatorial attitude. Culturally, no peculiarities of a sufficiently stable nature are apparent whereby any one race of streptococci may be sharply marked off from another. Gordon<sup>14</sup> has proposed the use of such fermentable substances as saccharose, inulin, raffinose, mannit, salicin, coniferin, etc. By their use he believes that he is able to say that this coccus is of salivary origin, that of fecal or pustular; in this way milk streptococci are separated from salivary races by their ability to ferment salicin. On this account Savage<sup>15</sup> regards this test as being of great value in identifying streptococci of bovine origin, but as there occur at times exceptions to this rule, the test, like many of a similar nature already known to us, can then be considered as a presumptive test only, if even that.

Other tests have been proposed and tried out, notably the hemo-

lytic, introduced by Schottmüller,<sup>16</sup> but have led to discordant results. Baumann<sup>17</sup> found that, with a series of pathogenic and non-pathogenic streptococci, the former were uniformly and markedly hemolytic, whereas the latter possessed no hemolytic power; amongst the non-hemolysers he classed the milk streptococci. Müller,<sup>18</sup> comparing the milk streptococci with those from pathological sources, noted that hemolysis occurred amongst both types, and regarded the test as one showing no differences of a sufficiently important nature. He quoted also from the literature in support of this view.

No better also are the tests in the field of agglutination. It is generally conceded that among pathogenic forms no reliable deductions as to possible identity of races can be drawn. Bergey<sup>13</sup> tested the method with a large number of races of milk streptococci using sera prepared from pathogenic varieties, but was unable to come to any satisfactory conclusions. Müller<sup>18</sup> obtained both positive and negative results among a series of pathogenic streptococci tested with pathogenic (homologous) sera, and in lesser degree among races of milk streptococci tested with the same sera. He concluded, however, that a positive result obtained with milk streptococci speaks strongly for the race being pathogenic, but a negative result leaves the question an open one. He is of the opinion that among the milk streptococci are some which are closely related to the pathogenic varieties. Baumann<sup>17</sup> and Savage<sup>15</sup> lay no stress upon agglutination as a means of differentiation, on account of its unreliability.

Tests of pathogenicity, too, are by no means a safe guide in determining the virulence of the races isolated. Beck<sup>19</sup> recorded that not a few of the streptococci isolated by him from the market milk of Berlin were possessed of considerable lethal power toward rabbits and guinea-pigs. Escherich, too, determined that certain races were pathogenic for white mice. But it is a well-known fact that not infrequently streptococci isolated from a variety of pathological sources in human disease produce most irregular results upon inoculation into laboratory animals, and similarly with milk streptococci we find that Reed and Ward<sup>3</sup>, Lammeris and van Harrevelt,<sup>20</sup> Brüning,<sup>21</sup> Seiffert,<sup>22</sup> and Bergey<sup>23</sup> (1901) record the statements that the organisms isolated by them were quite avirulent to their laboratory animals, chiefly rabbits and guinea-pigs. And here we must

agree with the statement of Rullmann and Trommsdorff,<sup>24</sup> speaking of this phase of the subject, that such a line of work argues nothing for the pathogenicity of these streptococci toward man, and our deductions must be drawn from another line of facts.

Despite, then, our inability to distinguish racial differences among the streptococci, we cannot but feel sure that there are present in the milk from time to time, cocci which undoubtedly have a virulence all their own toward the human species, and it is the belief of those who are competent to express an opinion that these cocci thus causing human infection are those giving rise to the acute contagious variety of mastitis, or *gelber Galt* of the Germans. Rullmann and Trommsdorff, however, are of the opinion that the presence of streptococci in freshly drawn milk, especially if accompanied with leucocytes, is a sign of a chronic mastitis and the milk is to be regarded with suspicion. In this they agree with the previous statements of Bergey.

The type of disease in human beings occasioned by an infection by such milk takes on two forms, the one marked by distinct and severe gastro-intestinal disturbances combined with general depression and malaise; the other form manifesting itself in a severe faucial angina, tonsillitis, a swelling or suppuration of the submaxillary or cervical glands, occasionally cellulitis, and well-defined constitutional disturbances. Such infections usually take the form of local epidemics confined to the region supplied by the infected milk. Petruschky and Kriebel<sup>25</sup> are of the opinion that the streptococci are responsible for much of the mortality in that form of disease known as summer diarrhea of infants. Examples of infection supposedly caused by these streptococci are given by Holst,<sup>26</sup> Stokes and Wegefarth,<sup>27</sup> Kenwood,<sup>28</sup> and Savage.<sup>29</sup> The latter states that, in an outbreak of sore throat in Colchester, he discovered a cow in the herd of the suspected dairy affected with acute mastitis, and upon removal of the sick animal the epidemic subsided; the milk of the animal contained enormous numbers of streptococci and pus cells. Under such circumstances, then, the discovery of the cause rests, not on bacteriological grounds alone, but most largely upon a clinical examination backed up by bacteriological and microscopical findings.

To face now the question of the importance of the presence of

streptococci in milk, we must acknowledge the correctness of the view that there exists in the udders of practically all normal cows a certain species of bacterium, in numbers greatly exceeding others, and that this bacterium performs a kindly service to the dairyman and is regarded as inoffensive. Then, too, we have to accept the facts recorded by many sanitarians that there are to be observed in the milks of different countries streptococci varying in quantity from 50 to 76.6 per cent of all bacteria present. But have we to subscribe to the interpretations so often attached to the finding of these bacteria, viz., that it is evidence that the cows giving the milk are diseased and that the milk is in consequence unfit for the use of the human subject? I think not. For if we did, then we would be bound to acknowledge that for the greater part milch cows suffer more or less continuously from inflammation of the udder, which is the inference drawn from reading the experiments of Bergey,<sup>13</sup> and Rullmann and Trommsdorff.<sup>24</sup> Kaiser,<sup>30</sup> and Savage,<sup>15</sup> on the other hand, both of whom have gone into examinations of milk and found streptococci to be present in most samples, have not cared to express an opinion in this matter.

The solution of the difficulty seems to me to be furthered if we choose to accept the views of Kruse,<sup>9</sup> Hölling,<sup>10</sup> and Heinemann,<sup>11</sup> namely, that the normal lactic acid bacteria of the udder are not bacilli, as most investigators have thought, but streptococci, and streptococci which, under the conditions in which we find them, are, as has been shown, for the most part non-pathogenic. Then, too, as to their origin, we might indulge in some profitable speculation. Steiger<sup>12</sup> states that there are three routes whereby an infection of the udder may take place: (1) direct infection through the teat-canals, constituting the galactogenous infection; (2) infection through wounds of the udder or teats, on the outside, by way of the lymphatic vessels, the lymphogenous infection; (3) the hematogenous infection, occurring in the course of a general infection having a local starting-point in some distant part of the body. Whatever, then, the mode of infection and the degree of infection produced (leaving aside the question of the acute contagious mastitis), might it not be assumed that after a time the cocci gradually part with their pathogenic powers, and, undergoing some modification, give themselves over to a sapro-



phytic existence, comparable to that led by bacteria in the mouths and intestines of the human subject? Again, it may not be necessary to imagine that an infection takes place according to any of the foregoing ways. It can as readily be conceived that, by a simple fouling of the teats in a "leaky" cow by dung or other dirt, growth of already saprophytic streptococci is permitted to occur along the teat-canal into the udder; or, in a non-"leaky" animal, by a similar style of infection directly after milking, when there yet remains within the orifices of the canals a sufficiency of milk to afford the proper conditions of growth.

That the foregoing arguments for the acceptance of the view that streptococci in milk have not the significance usually attached to their presence will satisfy or cover all points of criticism, I do not pretend to assume. The stand taken is simply put forward in the endeavor to bring into greater harmony facts that appear to be unnecessarily at variance and the cause of so much doubt, as well as of doubtful or seemingly divergent interpretations. The importance of the theme is not insignificant, and if the criticism engendered by this paper will lead to a careful reinvestigation of our facts, we may arrive, it is to be hoped, at no distant day to a much clearer point of view than is at present held regarding the status of the presence of streptococci in milk.

There yet remains, however, another question closely linked up with the preceding, and one, too, that on account of much divergence of opinion requires almost as great attention at the hands of investigators, and that is the presence of the so-called "pus cells," or, more properly, leucocytes in milk.

Too much has been made of this question upon what appears to me to be rather shallow grounds; and, in addition, the somewhat unfortunate choice of the term "pus cell," instead of leucocyte, is to be regretted. As Savage<sup>15</sup> remarks, "That milk should not contain pus cells few will deny, but what constitutes pus in milk? All milk contains leucocytes. When does a leucocyte become a pus cell, and what distinguishes one from the other?" Such is a well-merited challenge to the propriety of the use of the word "pus," as applied to the presence of white blood cells in milk. A decided expression of opinion upon this point is urgently needed, for it must be recognized

that the presence of leucocytes in milk up to a certain point is largely a physiological circumstance; beyond that, a pathological one. What are the bounds to be set in recognizing either of these conditions? Frankly, we do not possess sufficiently reliable data upon which to dogmatize. Bergey,<sup>13</sup> Doane,<sup>31</sup> Savage, and Rullmann and Trommsdorff<sup>24</sup> have demonstrated that in apparently healthy cattle cell numbers may vary among different cows, and in the four mammary quadrants of the udders. To what this variation is due is hard to say. Bergey, and Rullmann and Trommsdorff find that there is a proportional relationship between leucocytes and streptococci, a high leucocyte count usually being accompanied by a correspondingly high streptococcus count; but this state of affairs was not always found to be uniformly so. Savage, on the contrary, could not find any relationship whatever. Whether, apart from any signs of inflammation, chemiotactic substances play any part in leucocytosis due to the residence of saprophytic streptococci within the udder is not known; perhaps they do.

It is interesting to trace the attempts to put this question of leucocytosis upon a practical working basis. Apparently Stokes and Wegefath<sup>7</sup> in 1897 were the first to propose a plan for the estimation of leucocytes in milk, and with some modification their system is in vogue today in many laboratories in this country. Briefly it was as follows: Ten c.c. of a well-shaken sample of milk were measured out and centrifugalized in a hand centrifuge for five minutes, the supernatant fluid poured off, and a platinum loopful of the sediment spread over a slide, fixed by heat, cleared by ether, and stained with methylene blue and examined under an oil-immersion lens; the number of leucocytes were counted in 10 fields of the microscope, and if they ran over five cells per field, the milk was to be adjudged to contain pus. In 1899 Eastes<sup>31</sup> reported in an indefinite manner upon the presence of pus in milk. Bergey<sup>13</sup> in 1904, using the Stokes-Wegefath method, decided that if the number of cells were greater than 10 per field, it was indicative of the presence of pus. Stewart<sup>33</sup> used the sediment from 1 c.c. of milk and set the limit of cells at 22 per field. Slack<sup>34</sup>, using 2 c.c., spread the sediment over 4 sq.cm. of the surface of a slide, and allowed 50 cells per field of the oil-immersion lens as indicative of pus formation.

Writing in 1905 under the title, "The Doane-Buckley Method of Determining Leucocytes," Doane<sup>31</sup> criticizes the foregoing methods in these words: "There is but one element of scientific accuracy in the whole process. . . . the using of a definite quantity of milk"—which criticism seems to be largely justified. The later modifications differ only in the attempts to have a more or less definite area of the slide covered with sediment, which in fact adds little to the accuracy of the technique. Doane describes the technique of the Doane-Buckley method as follows: 10 c.c. of milk are centrifuged for four minutes in a graduated centrifuge tube at an approximate speed of 2,000 revolutions per minute; the cream is then lifted out with a cotton swab and centrifuged for another minute; the cream is again taken out as

before, and, without disturbing the sediment, the overlying milk is syphoned off, leaving fluid to the depth of  $\frac{1}{8}$ -inch above the sediment; two drops of a saturated alcoholic solution of methylene blue are now added, mixed thoroughly with the sediment, and set aside in boiling water for two or three minutes to allow the leucocytes to take up the stain; hot water is then poured in to fill up to the 1 c.c. mark on the graduated tube; the contents are now shaken vigorously and a portion taken up in a capillary pipette and placed upon the ruled surface of a Thoma-Zeiss blood counter and covered with the cover-glass; allowing a minute for the cells to settle down, a total count of all the squares is made, or, if the cells are very many, then an average of the whole number may be obtained by the counting of say 10 or 15 squares. Calculation of the cells per c.c. of the milk is then made. The fluid over the total squares represents 0.1 c.mm., or 0.0001 c.c. Then say that there were 50 cells counted in the whole ruled part of the slide, which is equivalent to 500,000 cells, but as this number came from 10 c.c. of milk, 1 c.c. contains 50,000 leucocytes. This method certainly is free of any criticism upon the grounds of lack of scientific accuracy, as it is plainly that method used by clinicians in the enumeration of the cells of the blood with slight modifications to suit the conditions.

Ward<sup>35</sup> upholds the accuracy of the Doane-Buckley method in an article published this year, stating that it gives much more satisfactory results than that of Stewart, the count running from 4 to 40 times higher.

Quite independently of Doane and Buckley, Savage<sup>15</sup> this year has practically worked out the same method, varying the details in some degree. He, too, felt the burden of the inaccuracy of the Stokes-Wegefath plan and so set about to develop one more suitable and more scientifically precise. As carried out by Savage, the process consists in taking 1 c.c. of the sample and putting it into a 20 c.c. graduated centrifuge tube, filling up to the 20 c.c. mark with Toisson's solution; after mixing well, the sample is centrifuged for 10 minutes, the cream is broken up thoroughly with a glass rod, and again whirled for 10 minutes. All the fluid is now removed but the last 1 c.c., which is stirred up well and the required amount is placed upon a Thoma-Zeiss counter and counted, the calculation of the cells being done by means of a special formula and rendered in cubic millimeters, thus making the counting part of the method apparently unnecessarily difficult, although its accuracy cannot be called into question.

Another system developed in Germany by Trommsdorff<sup>36</sup> and published this year differs in all respects from any of the foregoing, and although it is more accurate than the Stokes-Wegefath method, it is yet only capable of furnishing qualitative results, and not quantitative as should be the case, such as is afforded by Doane and Buckley's and by Savage's methods. In this method 5 c.c. of milk are taken and put in specially devised tubes, which are drawn out to capillary points and there carefully etched into divisions of 0.001 c.c., running from 0.001 to 0.02 c.c. in volume. Upon being centrifuged for a few minutes, the amount of sediment is read off and the amount calculated in volumes per 1,000 or 10,000 as desired. It can readily be seen that these results, qualitative as they are, may be invalidated by the presence of foreign matter blocking up the capillary tube, although the inventor claims that under his care the instrument is quite free from this objection. Its usefulness may possibly be further impaired by the accumulation of other material in the milk than pus, e.g., fibrin and mucin.

Of these several methods devised for the recognition of pus in milk only three merit any consideration, namely, those of Doane and Buckley, of Savage, and of Trommsdorff. In point of accuracy and true analysis, the first two methods and the last stand in parallel contrast with each other, exactly as do the two methods for the estimation of leucocytes in the blood, i. e., by actual count and by the hemato-krit; and of them, the one freer from error and more desirable is that by actual count.

To go into the results obtained by the authors would be to lengthen this paper very considerably. Suffice it to say that each finds much variation in the leucocyte count among individual cows, between the four mammary quadrants of each cow, and between individual cows' milk and that of the whole herd. As regards the question of standards, Rullmann and Trommsdorff consider that any sample yielding over 1 volume per mille is evidence enough that there is pus in the milk. To quote Savage,<sup>15</sup> "I cannot differentiate between a leucocyte and a pus cell, and I am not prepared at this stage to lay down an arbitrary standard as to what number of leucocytes per cb.mm. is to be designated *pus* in the milk." He also affirms that there is no relation between leucocytes and streptococci, so far as his figures go. Doane,<sup>31</sup> on the other hand, is more specific and states that a milk containing 500,000 cells per c.c., together with the presence of fibrin, is to be regarded as suspicious, while a content of 1,000,000 per c.c. associated with fibrin is conclusive of the presence of pus, i. e., evidence of mastitis.

This, then, closes the presentation of a subject that very evidently is at present in a rather chaotic state, but one that invites our further careful scrutiny, deliberation, and judgment, before it can be said that the milk problem is a settled one.

The following conclusions are offered:

1. It seems imperative to secure an early adjustment of the divergence of views of the taxonomic status of the so-called *B. lactis acidi*.
2. The statements of Kruse, Hölling, and Heinemann cast considerable doubt upon the value heretofore entertained regarding the significance of streptococci in milk.
3. It is not excluded by the evidence that pathogenic streptococci are to be found at times in milk; in fact, recorded observations make



this certain, the contamination arising from clinically recognizable cases of mastitis in the herds.

4. We are not as yet in possession of any reliable method for distinguishing a non-pathogenic from a pathogenic streptococcus.

5. The sanitary significance of the so-called "pus cell" has been greatly overrated. More scientific attention should be given to the study of the phenomena of lactic leucocytosis, together with a more accurate method of enumeration, such as that of Doane and Buckley, or of Savage.

6. Particularly, should more attention be given to veterinary inspection of the cows' udders, with less absolute dependence upon laboratory examination of milk for signs of infectious processes.

7. The time seems ripe for throwing open the whole question for discussion, and the framing of new rules to cover points raised and accepted.

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## LEUCOCYTE STANDARDS AND THE LEUCOCYTE CONTENT OF MILKS FROM APPARENTLY HEALTHY COWS.

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OF late years an increasing amount of attention has been given to the determination of the sanitary properties of milk supplies. The old standards, based largely upon the sense of smell and taste, are no longer regarded as the ultimate basis for a proper judgment as to the quality of milk. Various new methods have been introduced for the purpose of controlling the character of these supplies. Those so far proposed, however, have been developed for the detection of two different sets of conditions:

(1) The determination of the presence of organisms that have found their way into milk mainly through careless methods of handling.

(2) The detection of abnormal conditions in milk arising from its condition in the udder.

The first type of standards embraces those relating to the degree of cleanliness with which the milk is handled; the second concerns the healthfulness of the milk as it comes from the cow.

Among the more important standards that have been proposed may be mentioned, under Group (1), the following:

(a) The determination of the total germ content by cultural methods, a method which has been adopted by a considerable number of cities.

(b) The determination of the total germ content by direct microscopic examination, as proposed by Dr. Slack of the Boston Board of Health.

(c) The use of the Wisconsin curd test, by means of which is recognized the presence of gas-producing and taint-forming bacteria, organisms associated with careless methods of milk-handling.

Under Group (2) are included such test methods as reveal—

(a) The presence of special types of organisms, such as streptococci, that are presumed to be associated with the production of certain udder diseases.

(b) The supposed presence of pus, by the determination of pus cells, or the detection of associated elements, such as fibrin.

Each of these methods that have been suggested has elements of value, but in the present state of development, it is a question whether any absolute standard can be proposed that is a satisfactory basis for milk inspection. The actual detection of disease-producing organisms in milk is exceedingly difficult under most conditions. Fortunately there are not many diseases which occur in the cow that affect man. Tuberculosis is by far the most important of this class.

On the other hand, cattle not infrequently suffer from udder troubles—gargets of various types, in which inflammatory processes of varying degrees of severity may occur. In some cases these may be so slight as apparently not to affect the nature of the milk. Often, however, the milk becomes viscous, sometimes clotted or stringy, and may even be of a bloody nature. Occasionally such troubles as these develop into a gangrenous stage, in which pus is actually present in such quantities as to be easily recognizable. Milks of this character should, of course, be excluded from food supplies. In these cases, where a physical examination reveals an abnormal condition, a microscopic study naturally presents a far different picture from that which obtains in apparently normal milk. One of the prominent microscopic features in the case of milk that is abnormal at time of withdrawal is the presence of an unusually large number of certain blood elements, known as the white blood corpuscles, or leucocytes. In case pus is at all observable, these cellular elements are present in enormous numbers. Generally they can be detected, even where no physical signs of pus are apparent. The fact that milks known to be associated with abnormal conditions in the cow often contain these elements in such large numbers has led to the suggestion that all milks rich in leucocytes should be regarded with more or less suspicion.

Histologically, it is not an easy matter to differentiate between pus cells and the normal leucocytes, or white blood corpuscles. These latter cellular elements are, of course, normal constituents of blood, and are also found in variable quantities in milk supplies. It is quite probable that some of these cellular elements found in milk are derived from the gland tissues and are not leucocytes, although, upon staining, the majority of the cells are polymorpho-



nuclear. Thus far, emphasis has been placed on the quantitative rather than the qualitative relation of these cells in milk. Whatever may be their nature, they are at present considered as associated with pus, if they are found abundantly in the milk.

The presence of a large number of leucocytes, either in blood or milk, is regarded as indicating an irritation of an inflammatory character, but the important question is, what constitutes an abnormal number? How many leucocytes can be present in milk before it should be considered that suppuration has taken place? The standards, so far chosen, have been more or less arbitrarily selected on what appears as rather inadequate data, and, from a comparison of results, it is apparent that much more comparative work needs to be made. Even the technical methods by which the leucocytes are determined do not show harmonious and constant results, and the interpretations placed upon the data obtained vary still more.

The formulation of these standards for milk inspection has already led to much dissatisfaction and complaint on the part of the dairy interests. In a number of cases, even certified supplies which have been handled with great care have been rejected on account of the high leucocyte content found in them, although the closest veterinary inspection failed to reveal the presence of any udder abnormality. We quote from a recent correspondent in Tennessee:

The city chemist of Memphis is taking steps to condemn 35 to 75 per cent of the dairy cows of this county for alleged discovery of pus in the milk. He has condemned 16 out of 35 in our herd, and in the case of some dairymen, 90 per cent of their herds. The cows are in good condition and the veterinarian from the University of Tennessee has examined my herd, and pronounced their general condition good, and their udders perfect. There is absolutely no sign of inflammation.

In a letter from Doctor S. McC. Hamill of Philadelphia, who is the secretary of the Milk Commission of the Pediatric Society of that city, he says:

Some of the best herds have been publicly condemned and brought into disrepute. It has not been shown by anyone that milk high in leucocytes has any injurious effect, nor has it been shown that their presence meant *per se* inflammatory changes in the udder.

As secretary of this commission, Doctor Hamill enters a vigorous protest against the attitude of the city board of health, which is enforcing a standard whereby milk supplies are condemned in which are found 100,000 or more leucocytes per c.c.

## METHODS OF EXAMINATION.

Before discussing the standards proposed it is pertinent to study the methods of examination which so far have been devised.

Naturally the number of blood elements can best be determined by the use of the centrifuge. In the main, two methods have been suggested by which the leucocytes can be quantitatively determined; viz.:

(a) *Smear-sediment method*.—This method, devised by Stokes<sup>1</sup> of the Baltimore Board of Health, and later somewhat modified by Stewart of the Philadelphia Bureau of Health, consists in centrifugalizing a definite quantity of milk in rubber-stoppered tubes, pouring off the cream and milk, rubbing up the collected sediment with water, and smearing this emulsion on a glass slide of definite dimensions. The preparation after staining is examined by a one-twelfth inch oil immersion lens, and the number of leucocytes per field noted.

(b) *Direct quantitative method*.—This method was suggested by Doane<sup>2</sup> and is carried out in essentially the same way as the generally accepted quantitative method of determining the blood cells. Ten c.c. of milk are centrifugalized in sedimentation tubes. The cream is then removed by means of a cotton swab and the material treated in the same manner a second time, thus wholly eliminating the fat.

The supernatant milk is then siphoned off. Water is added to the collected sediment, bringing the same to the one c.c. mark, and the whole thoroughly emulsified. Quantitative determinations are made from this material in a ruled blood counter (Thoma-Zeiss pattern).

Savage<sup>3</sup> has recently studied the relation of streptococci and leucocytes in milk and has employed a method quite similar to that of Doane. His technique was evidently developed independently, as he refers only to Stokes' method in discussing the subject.

Stokes' method has been adopted by most investigators who have studied this question. Bergey at Philadelphia has carried on quite an extensive series of examinations by this method, except that he substituted a cover-slip for the glass slide of given dimensions. The health boards of Philadelphia and Boston, the two cities that have been the most active in the examination of milks as to leucocytes, have both adopted this method.

The objections to this technical procedure are quite apparent. It is impossible to remove wholly the sediment from the rubber corks, and it is quite out of the question to distribute this material uniformly enough over the glass surface so that duplicate specimens can be secured which will give consistent results. This distribution can be more effectively obtained by emulsifying the sediment with a small quantity of water. Moreover, this treatment requires staining the cells, and if the leucocytes are not spread in an exceedingly thin layer, it is impossible to count them accurately. In our experiments there appears to be but little question as to the comparative merits of the two types of methods that have been proposed.

<sup>1</sup> "The Microscopic Examination of Milk," *Jour. of State-Med.*, 1897, 5, p. 439.

<sup>2</sup> *Bull. 102, Maryland Agri. Expt. Sta.*, 1905 (often referred to in the literature as the Doane-Buckley method).

<sup>3</sup> *Jour. Hyg.*, 1906, 6, p. 123.

## COMPARISON OF METHODS OF EXAMINATION.

In order to determine their relative accuracy, a considerable number of duplicate examinations were made upon the same sample of milk by the Doane-Buckley and the Stokes-Stewart methods. These data are incorporated in Table 1, from which it appears that

TABLE 1.

COMPARATIVE EXAMINATION OF LEUCOCYTES PER C.C. IN MILK, AS DETERMINED BY THE TWO METHODS PROPOSED.

VOLUMETRIC (DOANE-BUCKLEY) METHOD					SMEARED-SEDIMENT (STEWART) METHOD			
Sample	First Examination	Second Examination	Average	Percentage Variation	First Examination	Second Examination	Average	Percentage Variation
1.....	590.625	700.000	645.310	18.0	403.200	238.500	320.850	69.0
2.....	325.000	288.750	306.875	12.0	16.200	113.400	64.800	587.0
3.....	1,012.500	975.000	993.750	3.7	99.100	154.000	126.500	55.4
4.....	11.250	12.500	11.875	11.1	4.500	16.200	10.360	260.
5.....	701.250	687.500	694.375	2.0	82.870	146.830	114.850	77.1
6.....	146.250	155.000	150.625	5.6	136.020	120.700	128.360	12.6
7.....	20.000	20.000	20.000	0.0	19.810	18.900	19.370	4.7
8.....	193.750	187.500	190.625	3.3	153.900	111.700	132.800	37.7
9.....	881.250	901.250	891.250	2.2	366.300	517.500	441.900	41.2
10.....	50.000	50.000	50.000	0.0	0	9.000	.....	.....
11.....	42.500	51.250	46.875	20.0	63.000	76.560	69.810	21.4
12.....	413.750	422.500	418.125	2.0	135.210	150.430	142.820	11.2
13.....	10.000	10.000	10.000	0.0	2.250	13.510	8.880	500.0
14.....	438.750	425.000	431.875	3.2	193.670	145.930	169.800	32.5
15.....	125.000	132.500	128.750	6.0	0	17.115	.....	.....
16.....	4.680	3.900	4.290	10.0	1.350	0	.....	.....
17.....	87.500	90.000	88.750	3.0	40.530	9.000	24.760	350.4
18.....	543.750	537.500	540.625	1.1	284.650	152.235	218.440	87.0
19.....	443.750	443.750	443.750	0.0	275.640	276.545	276.095	0.3
20.....	222.500	231.250	226.875	4.3	126.110	159.440	142.775	26.4
21.....	245.000	247.500	246.250	1.0	174.750	202.680	188.720	16.0
22.....	1,076.250	1,057.500	1,066.875	1.7	439.590	545.885	492.740	24.2
23.....	142.500	125.000	133.750	14.0	71.180	87.375	79.280	22.7
Average, 5.6%					Average, 112%			

the results obtained by the volumetric method (Doane-Buckley) are very much more harmonious than those secured by the smeared-sediment method. The average percentage variation in duplicate examinations in the smeared-sediment method is very great (112 per cent), while that of the other method rarely ever exceeds more than 10 to 20 per cent, and in 23 tests averaged less than 6 per cent.

It is noteworthy in the above that, quantitatively, higher results were almost uniformly obtained by the Doane-Buckley method than by the Stokes-Stewart method. This fact should be taken into consideration in the application of any standard.

Ward has also recently reported a comparison of these two methods,<sup>1</sup>

<sup>1</sup> *Rep. California State Board of Health, 1905-1906, p. 142.*

in which similar results were obtained. From these data it appears, without question, that the volumetric method is very much more accurate for the determination of the actual conditions in milk; and not only in scientific studies on this question, but also in the practical application of any standard in public health work this should be given preference over the smeared-sediment method.

#### TECHNIQUE USED IN WORK HERE REPORTED.

The method which we have followed in the work to be reported is essentially the Doane-Buckley method with some slight modifications. A turbine Babcock tester was employed to centrifugalize 10 c.c. of milk in ordinary sedimentation tubes twenty minutes at 1,200 revolutions per minute. We have not followed the practice of re-mixing the cream and again centrifugalizing, as our experience showed so few leucocytes in the cream as to be practically negligible. Savage<sup>1</sup> found that the number of leucocytes in the supernatant fluid did not exceed 12 per cent of the total count.

The cream and the supernatant milk were removed, with the exception of the last half cubic centimeter, by aspirating with an exhaust pump, and wiping the walls of the tube with a cotton swab. The thoroughly mixed residue was then placed in the blood counter and examined unstained. While most observers have usually stained this material prior to examination, we have found no difficulty in working with unstained material. The cellular elements are easily differentiated, as they settle quickly to the bottom of the cell. The fat, which is a disturbing factor in such microscopic observations, is practically all removed in this process; the very few small globules which remain rise to the top of the liquid in the counting cell and do not interfere with microscopic observations.

The count is made with a No. 1 eye-piece, and a  $\frac{1}{8}$ -inch objective. Where the number of leucocytes exceeded 12 or 15 per microscopic field, six sets of 16 each of the smallest ruled squares of the blood counter, or approximately one-fourth of the entire area of the ruled scale, were counted. Where it was less than this, 10 to 16 such sets were counted. The average was then obtained, which, when multiplied by the factor 12,500 (the ratio between the counted volume and one c.c.), gave the total number of leucocytes per c.c.

#### FORMULATION OF STANDARDS AS TO PUS CONTENT.

Various numerical standards have been suggested from time to time as indicating the presence of pus in milk. A comparison of these shows that considerable variation exists, not only in the standards themselves, but in the interpretation of results.

Stokes<sup>2</sup> reached the conclusion that pus was present when more than ten<sup>3</sup> cells were found in a  $\frac{1}{12}$ -inch field. Bergey<sup>4</sup> adopted this same standard (10 per field)

<sup>1</sup> *Loc. cit.*, p. 129.

<sup>2</sup> *Rep. Health Department, Baltimore, 1890*; also *Med. News*, 1897, 71, p. 45.

<sup>3</sup> Since then, according to Doane (*Bull. 102, Maryland Agri. Expt. Sta.*, p. 214), Stokes has suggested the advisability of increasing this limit to 25 leucocytes for the same field.

<sup>4</sup> *Univ. of Pa. Med. Bull.*, July-Aug., 1904, p. 2.



in 1900,<sup>1</sup> and his later studies have confirmed his opinion as to the correctness of this standard.

Stewart<sup>2</sup> of Philadelphia has increased his standard considerably above the limits selected by Bergey. He recommends spreading the sediment over one sq. cm. and advises condemnation of the sample when the microscopic field (with  $\frac{1}{12}$ -inch objective and No. 3 eye-piece) contains more than 23 leucocytes per field. As it requires practically 4,400 fields to cover the one sq. cm. of the smeared preparation, this would be equivalent to 100,000 leucocytes per c.c. This standard has been quite widely accepted and is now in force in a number of cities.

Doane, using his volumetric method, suggested a materially higher standard as he found quite frequently that the milk of apparently healthy animals exceeded the 100,000 limit. He suggested 500,000, and possibly 1,000,000 leucocytes per c.c., as a preferable limit.

As pointed out above, and also by Ward, the results obtained by the Doane-Buckley method show on the same sample of milk a very much higher leucocyte content than those obtained by the smeared-sediment method. It is, therefore, very necessary that a uniform method of examination be selected before adopting any standard for comparison.

#### RESULTS OBTAINED IN PRACTICAL WORK ON MILK EXAMINATION.

For the last two years certain milk supplies received in Philadelphia and Boston, have been examined as to numerical leucocyte content, the presence of streptococci, and total bacterial content.

Stewart has investigated six of the best milk supplies furnished in Philadelphia—supplies that were certified by the Milk Commission of the Pediatric Society of that city, with the following results:

TALBE 2.

RESULTS OF EXAMINATION OF CERTIFIED MILK SUPPLIES OF PHILADELPHIA BY LEUCOCYTE STANDARDS (STEWART).

Herd Number	Total Number of Samples Examined	Number of Samples containing Pus in Excess of Legal Standard (100,000 per c.c.)	Percentage of Samples Condemned on Pus Standards
1.....	54	7	13
2.....	16	4	25
3.....	54	2	4
4.....	54	4	8
5.....	52	4	8
6.....	52	0	0

Slack (*Thirty-fourth Health Report*, Health Department, Boston, 1905) gives the results of an inspection of the regular supplies furnished Boston, in which 5,559 samples were tested, and 279 (or 5 per cent) of them were condemned as containing

<sup>1</sup> Reference to Bergey's original paper, published in the *Report of Secretary of Agriculture of Pennsylvania, 1900*, p. 123, shows that he proposed a limit of five leucocytes per  $\frac{1}{12}$ -inch field, and any excess of this number he believed to be associated with a pathological condition of the udder, especially if the leucocytes showed a tendency to be bunched together.

<sup>2</sup> *Amer. Med.*, 1905, 9, p. 486.

pus in such quantities as to be considered as infected. Presumably this condemnation was made on the basis of an excess of 100,000 leucocytes per c.c., although specific information as to this point is not presented. In this case, the method of examination was the slight modification of that used by Stokes and Stewart.

Savage, using the direct quantitative method of enumeration, examined the milk of 11 herds, or a total of 40 cows, in which in no case was there any physical symptom of udder infection. The range in leucocyte content found by him was 35,000 to 4,380,000 per c.c. Thirty-three of the 40 cows had 100,000 leucocytes per c.c., or over. An examination by him of the milk supplies taken from 17 different herds showed a range from 21,000 to 1,980,000 leucocytes per c.c. All but three herds showed a leucocyte content in excess of the 100,000 limit.

Doane has made a careful study by quantitative methods of the Station herd at the Maryland Experiment Station. Twenty-five animals were examined three times during a period of about three months. The first examination showed an excess of 100,000 leucocytes per c.c. in 14 out of 23 cases; the second examination, in 17 out of 22; and the third (two months later), in 10 out of 16 cases.

Another well-kept dairy herd near Baltimore was also examined by him and, in a single test made on 102 cows, the leucocyte content ranged from 2,000 to 4,600,000 per c.c., with an average of over 240,000. Forty-six animals in this herd showed an excess of 100,000.

It would appear from these figures that the milk of healthy animals often contains a much larger number of leucocytes than has heretofore been considered as permissible in a wholesome milk supply, but it is highly desirable that further data be accumulated, especially on individual cows whose physical condition could be more closely controlled.

#### RESULTS ON UNIVERSITY HERD.

No study has yet been reported on milks taken from individual animals where the examination has extended throughout a considerable consecutive period of time. Data of this sort would seem to be necessary in order to secure the normal individual range in leucocytes. The examinations which are here reported were made on samples taken from the University herd, which is composed of high-grade and pure-bred animals of representative dairy breeds. Daily examinations from the mixed-pail milk for a period of about one month were made during the summer (June and July), and then on the same animals for about two months in the fall (October and November).

This herd has been given a much closer degree of supervision than is ordinarily the case. The cows were carefully handled as to feeding, and also subjected to frequent veterinary inspection. The herd, with but a single exception, was in apparently good condition. This cow (No. 3) had chronic mammitis in one quarter.

TABLE 3.  
LEUCOCYTE CONTENT OF MILK OF INDIVIDUAL COWS IN UNIVERSITY HERD (000 OMITTED).

No. of Animal	Condition of Animal	JUNE, 1906						JULY, 1906						SEPTEMBER, 1906					OCTOBER, 1906					REMARKS				
		24	25	26	27	28	29	2	5	12	13	15	16	17	19	24	25	26	27	28	1	2	3		4	5	8	
2.....	Normal	37	128	.....	.....	.....	131	.....	64	31	.....	29	31	35	51	.....	144	.....	60	99	139	135	.....	200	194	.....	In heat July 15, '06	
8.....		.....	.....	5	.....	.....	16	.....	22	1	.....	.....	10	25	64	39	16	26	19	12	10	.....	.....	9	.....	46		
9.....		.....	.....	1,097	.....	.....	306	297	.....	260	382	.....	501	176	320	426	322	562	504	270	197	.....	.....	.....	.....	444	Calved June 23, '06	
10.....		.....	39	.....	.....	.....	60	.....	.....	4	.....	26	22	26	232	204	74	102	212	144	87	.....	.....	.....	.....	72	Calved September 14, '05	
11.....		.....	115	.....	.....	.....	162	.....	.....	139	.....	120	54	57	131	160	64	76	87	90	132	.....	.....	.....	.....	57	Calved May 3, '06	
12.....		.....	.....	60	.....	.....	.....	37	.....	.....	35	.....	12	12	44	50	64	72	216	15	27	11	.....	.....	35	106		
14.....		.....	272	.....	208	.....	137	.....	204	.....	439	372	.....	456	200	.....	550	629	644	531	.....	175	.....	201	189	.....	Calved April 5, '06	
15.....		.....	.....	.....	159	.....	224	.....	370	.....	141	297	.....	306	129	254	304	341	137	132	.....	.....	.....	.....	.....	.....		
16.....		.....	103	61	.....	47	.....	.....	.....	55	200	67	.....	44	82	201	41	.....	126	65	216	.....	.....	50	117	57		
19.....		.....	896	.....	1,026	.....	35	.....	1,707	.....	314	1,100	.....	397	641	806	.....	.....	.....	.....	.....	.....	.....	551	595	235	Gargety for a time in 1905	
20.....	.....	.....	.....	122	.....	.....	45	87	.....	95	156	.....	187	141	129	401	85	130	106	208	95	50	.....	110	141	151	Calved October 1, '06	
22.....	.....	.....	82	.....	.....	.....	.....	56	.....	19	76	.....	79	16	41	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....			
24.....	.....	.....	181	.....	.....	186	.....	92	.....	214	125	.....	297	39	141	64	85	82	60	72	.....	141	.....	.....	.....	87	Milk fever June 12, '06	
26.....	.....	.....	4	.....	23	82	.....	.....	.....	.....	.....	7	12	6	.....	35	31	8	25	29	21	14	.....	.....	.....	42		
27.....	.....	58	101	.....	80	59	.....	81	.....	350	.....	164	329	194	157	731	419	381	401	714	.....	1,594	1,056	1,000	.....			
28.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....			
29.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....			
1.....	Slight induration Bad; acute mammitis Udder coarse and hard	11	.....	115	124	.....	50	.....	350	162	.....	226	162	235	539	.....	144	.....	60	99	139	135	.....	200	194	.....	Calved August 21, '06	
3.....		.....	.....	36	.....	.....	16	.....	32	32	.....	51	25	57	56	107	206	172	125	145	2,267	.....	.....	1,287	1,104	669	Calved July 7, '06	
4.....		.....	495	.....	21	.....	564	.....	609	.....	.....	229	501	.....	329	435	722	904	212	1,039	1,420	.....	.....	.....	.....	607	Aborted March 10, '06	
5.....		Slight induration	932	.....	205	.....	222	.....	.....	645	320	694	.....	.....	135	257	682	297	282	316	797	166	.....	.....	.....	.....	275	Calved May 2, '06
6.....		Indigestion	304	544	.....	.....	.....	.....	214	.....	275	75	.....	194	157	191	210	187	169	100	497	.....	157	.....	160	.....		
7.....	Slight induration	.....	.....	60	.....	77	.....	63	.....	79	60	.....	81	56	41	66	51	.....	72	66	11	229	.....	.....	.....	10	Calved March 17, '06	
13.....	.....	.....	47	.....	39	.....	32	41	20	10	.....	45	107	22	90	541	831	350	244	245	36	102	49	630	1,101	320		
17.....	.....	.....	.....	270	1,367	707	.....	1,281	1,482	.....	325	.....	.....	.....	1,162	854	326	566	395	972	150	410	.....	.....	1,260	.....	Calved April 26, '06	
18.....	.....	110	427	.....	227	134	.....	.....	312	.....	464	.....	357	795	417	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Calved October 3, '06. Had milk-fever soon after	
21.....	.....	.....	468	.....	.....	.....	.....	326	.....	185	.....	331	1,581	200	205	106	175	82	74	189	.....	.....	.....	.....	.....			
23.....	.....	.....	.....	75	1,132	.....	600	.....	657	380	.....	1,085	864	441	600	379	197	94	144	304	100	.....	.....	441	154	.....		
25.....	.....	1,068	.....	.....	133	.....	.....	.....	290	451	616	.....	81	129	254	79	139	.....	56	102	32	67	30	.....	.....	87	Calved September 18, '06	

No. of Animals	Condition of Animal	OCTOBER, 1906																			NOVEMBER, 1906			REMARKS			
		9	10	11	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31 A. M.	31 P. M.	1		2	3	
2	Normal	.....	.....	156	92	.....	172	135	86	147	75	32	121	59	77	212	157	76	222	243	229	162	210	170	404		
8	"	11	9	0	21	5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		
9	"	941	472	576	.....	1,066	712	1,037	400	387	126	864	307	241	344	560	1,060	525	262	750	531	310	791	491	564		
10	"	45	22	166	44	69	339	150	81	120	90	64	160	67	70	65	36	60	145	69	91	157	166	54	162		
11	"	20	61	47	20	57	55	35	101	50	90	50	111	100	137	102	55	137	77	41	71	80	79	77	107		
12	"	.....	.....	.....	70	26	50	75	145	46	87	132	90	80	65	182	150	117	82	172	124	157	105	117	172		
14	"	345	.....	.....	.....	.....	339	892	304	262	302	597	301	169	419	222	276	341	610	229	291	204	300	104	164		
15	"	.....	.....	6	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		
16	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		
19	"	381	307	275	230	204	347	557	370	581	264	185	264	1,066	347	610	366	294	1,214	581	481	335	681	512	614		
20	"	.....	139	254	69	139	212	401	175	91	166	11	137	131	216	130	297	231	132	169	412	97	162	176	231		
22	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		
24	"	147	110	187	54	181	162	232	170	135	75	52	46	169	114	136	92	99	137	91	125	61	50	92	115		
26	"	30	36	112	30	47	24	30	19	21	21	27	12	12	22	42	24	12	20	41	26	41	46	30	21	In heat October 12 and October 30, '06	
27	"	350	2,101	1,416	1,087	2,014	.....	30	39	30	42	201	34	36	74	44	110	35	17	61	76	54	44	142	46	36	
28	"	.....	.....	.....	.....	.....	24	47	57	32	12	15	15	16	19	15	19	12	21	19	73	20	49	27	37		
29	"	.....	.....	.....	.....	.....	12	25	14	24	41	41	12	40	17	10	26	10	30	11	15	1	19	20	20		
30	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		
1	"	.....	.....	156	92	.....	170	189	89	175	99	182	70	260	175	151	187	210	107	250	104	150	125	142	162		
3	Slight induration	.....	489	912	3,475	3,195	3,925	1,097	1,501	1,350	1,044	1,041	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		
4	Bad; acute mammitis	237	304	3,045	4,952	205	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		
5	Udder coarse and hard	302	312	122	407	604	412	785	689	1,781	581	245	494	432	279	389	1,150	354	395	354	237	351	281	390	976		
6	Slight induration	.....	.....	225	179	220	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		
7	Indigestion	11	57	20	.....	.....	17	231	41	42	82	30	17	37	66	100	30	26	137	54	62	65	106	45	79		
13	Slight induration	107	.....	62	294	172	160	66	41	15	501	1,157	970	654	232	275	40	52	49	.....	51	30	46	25	55		
17	"	1,420	1,676	1,750	4,232	514	1,200	1,487	591	872	595	1,166	985	3,650	841	1,329	1,281	1,266	2,451	3,627	822	510	397	494	1,225		
18	"	.....	1,101	1,801	100	182	80	122	87	104	160	107	94	156	67	75	34	32	115	110	81	101	170	87	120		
21	"	.....	.....	147	50	145	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		
23	"	62	414	172	1,595	704	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....		
25	"	9	.....	144	50	50	104	84	44	132	39	62	46	62	65	60	51	31	172	135	185	115	350	110	125	Gargety during October, '06	





In order, however, to ascertain with especial care the exact condition of each animal, as to the quality and condition of her udder, the entire herd was subjected to a most rigid examination by Dr. Alexander, the Station veterinarian, and Professor Humphrey, of the Animal Husbandry Department. In this examination every abnormality, however slight, was taken into consideration. We have therefore divided the results reported below on the basis of this physical examination, including in one group all those animals which were wholly free from any udder blemish whatsoever, while in the second group are included those in which some manifestation of present or past udder trouble was observable. In this latter group, the induration often was very slight, but the previous history of the animal usually indicated that such animals had had some form of udder disturbance at some earlier date. At the time of the present examination the milk of these cows showed no abnormal condition, except in the single case mentioned. This history is especially interesting, as it may throw some light on the cause of the high leucocyte content of apparently healthy cows.

The detailed results are herewith presented in Table 3 in which the daily observations are recorded in full, so that the same may be critically studied by other observers. These data can be comprehended in their entirety more readily, in graphical, rather than tabular, form, and in Fig. 1 they are therefore reproduced.

In Table 4 is condensed a summary of the foregoing results, in which the maximum and the minimum, as well as the average leucocyte content, is given; also the various observations are grouped as to the respective number of times in which the leucocyte content came within certain specified limits.

In discussing these results the herd will be separated into two sections:

1. Cows which show an entire freedom from all udder troubles, either past or present.
2. Cows showing upon careful physical examination a slight induration or otherwise abnormal condition of the udder, but no unusual condition as to character of their milk supply.

Of the 30 animals in the herd, only one (No. 3) at the time these examinations were made showed any symptoms of active udder

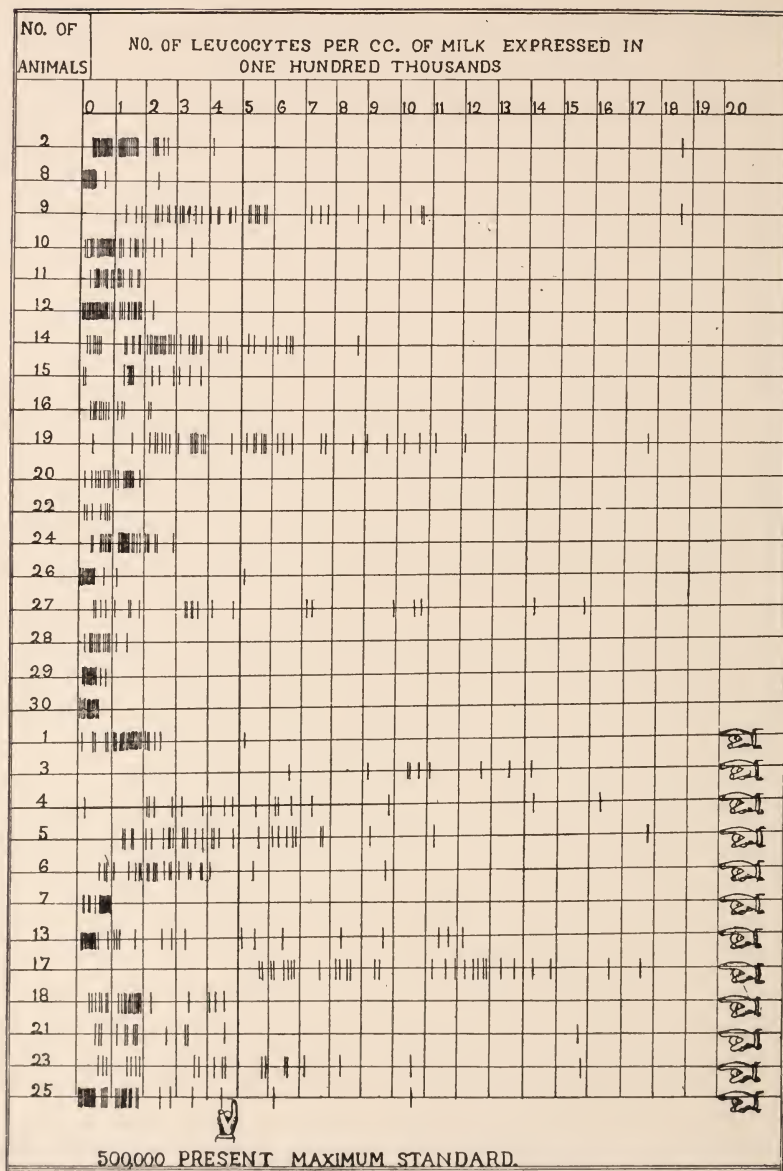


FIG. 1.—Leucocyte content of milks from University herd. Each determination represented by short vertical line. Animals marked with hand showed udder abnormalities. Note relative proportion of cases in excess of the present maximum standard.

trouble. In this case the milk was bloody and often clotted. The milk of the remaining 29 animals was perfectly normal in every respect, not only on the basis of its physical appearance, but by chemical and bacteriological analysis as well.

TABLE 4.  
SUMMARY OF DATA IN TABLE 3.

Group 1. Perfectly Healthy Animals.

No.	CONDITION	No. OF TESTS	MAXIMUM COUNT	MINIMUM COUNT	AVERAGE OF ALL TESTS	No. TIMES LEUCOCYTE CONTENT PER C.C. FELL WITHIN FOLLOWING LIMITS				
						50,000 or Below	From 50,000 to 100,000	From 100,000 to 500,000	From 500,000 to 1,000,000	1,000,000 and Above
2	Normal	38	403,750	26,250	121,175	8	10	20	..	..
8	"	20	63,750	1,250	18,815	10	1	..	..	..
9	"	38	1,060,250	126,250	536,280	..	..	21	13	4
10	"	38	338,750	3,750	100,400	8	15	14	..	..
11	"	38	162,500	20,000	85,130	5	19	14	..	..
12	"	36	216,250	11,250	85,225	11	12	13	..	..
14	"	37	802,500	137,500	327,000	..	..	29	8	..
15	"	12	341,250	128,750	235,520	..	..	12	..	..
16	"	16	216,250	41,250	96,360	3	7	6	..	..
19	"	30	1,707,500	35,000	561,825	1	..	17	13	5
20	"	41	412,500	11,500	162,500	2	8	31	..	..
22	"	7	81,850	16,250	52,770	3	4	..	..	..
24	"	30	207,000	38,750	121,300	2	16	21	..	..
26	"	30	532,500	3,750	42,000	36	1	1	1	..
27	"	23	2,101,250	58,000	652,060	..	4	10	2	7
28	"	24	201,250	16,250	60,130	14	7	3	..	..
29	"	20	72,750	3,750	25,000	29	2	..	..	..
30	"	26	46,250	31,250	19,880	26	..	..	..	..
Totals .....		537	.....	.....	.....	167	106	212	37	16
Percentage of total .....		...	.....	.....	.....	31.1%	19.8%	39.4%	6.8%	2.9%

Group 2. Animals Having Udder Indurations of Varying Severity but Producing Apparently Normal Milk (Except No. 3).

1	Slight induration	38	538,750	11,250	167,725	1	7	29	1	..
3	Bad; chronic mammitis	27	3,625,000	16,250	903,380	5	3	6	2	11
4	Udder coarse and hard	19	4,952,500	21,300	972,700	1	..	8	6	4
5	Slight; one-quarter	39	1,781,250	122,500	476,520	..	..	27	10	2
6	Indiges.; normal otherwise	19	543,750	75,000	213,700	..	2	10	1	..
7	Slight; one-quarter	38	231,250	7,500	63,375	15	17	6	..	..
13	One-quarter harder	43	1,101,250	10,000	225,935	18	6	11	6	2
17	Slight; one-half of udder	40	4,232,500	150,000	1,274,115	..	..	8	13	19
18	One-quarter slight (?)	32	1,801,250	32,500	249,450	2	7	21	..	2
21	One-quarter harder	15	1,581,250	66,850	304,125	..	3	11	..	1
23	Slight in one-quarter	22	4,132,500	62,500	630,170	..	3	10	6	3
25	One-quarter affected	30	1,068,000	8,750	149,950	7	13	17	1	1
Totals .....		371	.....	.....	.....	49	61	170	46	45
Percentage of total .....		...	.....	.....	.....	13.2%	16.4%	45.9%	12.4%	12.1%

RESULTS.

A study of the preceding tabular data shows some peculiar results. It is apparent that the leucocyte content of some of the cows was remarkably uniform, while in a considerable number of cases the

results were widely variable. It is to be noted, however, that the more uniform results were found in the first section of the herd, in which no previous history of udder trouble was observed. Of the 18 out of the 29 animals which showed a leucocyte content that was quite constant on successive examination, 14 cases belonged to Group 1 and only four to Group 2. Of the 11 instances in which the leucocyte content was subject to wide fluctuations, only four belonged to Group 1 while seven were found in the second group in which there was a more or less marked induration in the udder.

This would seem to indicate that lower and more constant results are more likely to be obtained with animals in which no udder trouble had ever appeared than with those having any history of a past inflammatory condition.

It is of course hardly possible to average results that fluctuate so widely, and draw any satisfactory conclusions, but if the data are arranged, as in Table 4, on the basis of the number of times the leucocyte content fell into certain numerical groupings, it is evident that the cellular content of milks drawn from perfectly normal cows averages lower than in Group 2.

Doane has suggested 500,000 leucocytes per c.c. as a possible limit by his method. On the basis of this standard, the results of our examination would be as follows:

Eighteen animals in the perfectly healthy group showed in 537 tests a leucocyte content of 500,000 or less in 90 per cent of the cases, while the 11 animals, in which induration was more or less marked, in 371 tests showed a similar condition in 75 per cent. While it appears from this that milks of high leucocyte content were found with considerably greater frequency (approximately two and one-half times), in the case of cows showing a fibrous induration of the udder, still it is noteworthy that practically one-tenth of all examinations made on cows entirely normal and healthy in all respects showed a leucocyte content that was in excess of the highest standard yet established. In this group of perfectly healthy animals 16 cases (or 3 per cent) were observed in which the cellular content was in excess of even 1,000,000 cells per c.c.

In the second section of the herd two animals contributed 30 of the 45 observations in which more than 1,000,000 leucocytes per c.c. were found. One of these (No. 3) was the only animal in the herd



that showed any acute udder trouble. No. 17, while showing no marked involvement of the udder, almost invariably had a high leucocyte content.

When these results are studied on the basis of whether they would pass the standards that have been proposed as a maximum limit, it appears that in many cases they would have been condemned. While it is impossible to compare accurately the results of the examination by the direct quantitative method with what would have been found by the smeared-sediment method, it is certain that a considerably higher standard would be necessary, if the Doane-Buckley method was the one in general use.

It is apparent from these studies that the leucocyte content of normal milk drawn from apparently normal animals is quite often so high that the milk would be classed as coming from diseased animals when judged by the standards that have heretofore been proposed. That such results are obtained from animals whose record has shown no clinical history of a diseased condition would signify that complete reliance cannot be placed upon quantitative leucocyte standards alone.

While it is undoubtedly true that cows suffering from udder trouble often do produce milk that is rich in leucocytes, it would seem equally true that similar quantitative results were found with such frequency in milks from perfectly healthy animals as to vitiate the accuracy of a test based solely on a numerical foundation. This being true, it seems necessary to modify the limits that have been adopted by a number of cities where the acceptance or rejection of a milk supply is based upon such standards as are here considered. As interpreted at present, they undoubtedly work injustice to the dairy interests, as milk supplies would be frequently condemned which come from animals in which no clinically recognized troubles can be ascertained.

Undoubtedly the leucocyte standard can be so formulated as to be of material service in the matter of milk inspection, but the question needs further study. So far as technique is concerned, the volumetric method (Doane-Buckley) is the more accurate. As to its applicability in routine inspection work, our experience is that this test can be made as rapidly as the smeared-sediment method, and is less trying on the eyes.

## THE COMPARATIVE VALUE OF BACTERIAL AND TEMPERATURE REGULATIONS FOR A CITY'S MILK SUPPLY.

FRANCIS H. SLACK.

*(From the Boston Board of Health Laboratory, Boston, Mass.)*

IN May, 1904, Boston established a bacterial regulation requiring market milk to contain not more than 500,000 bacteria to the c.c., and a temperature regulation requiring the milk to be kept below 50° F. Since these regulations were established simultaneously, it is impossible to give the effect of each singly in improving the milk supply; but a comparison of temperatures and bacterial counts on the same samples is instructive, and gives an insight into the relative values of the two regulations, each of which is shown to be important.

This paper is based on a total of 11,403 samples taken during three years, from June 1, 1904, to November 1, 1906. During this period both temperatures and plate counts were taken on 8,589 samples. On the remaining 2,814 samples temperatures were taken, but no counts were made, because they were passed as within the limit for bacteria on the microscopic examination alone; the error of this latter method with a careful worker is practically negligible as shown in a previous paper read before this Association.<sup>1</sup> (Of the 2,814 samples so passed 89 per cent were also within the temperature regulation.)

The condition of a milk supply untrammelled by bacterial or temperature regulations may perhaps be approximated by a consideration of the data of the first seven months' work in Boston after the regulations were made and while the work was educational in its character, warning notices alone being sent to those who failed to meet the conditions. The double examination, i. e., bacterial count and temperature, was made on 3,043 samples seized during these 7 months. The results of these examinations are as follows: 54 $\frac{1}{4}$  per cent were within both standards; 24 $\frac{1}{4}$  per cent had high temperatures with low bacterial content; 12 per cent had high bacterial content with low

<sup>1</sup> *Rep. and Papers, Amer. Pub. Health Assoc.*, 1905, 31, Pt. 2, p. 214.

temperatures and  $9\frac{1}{2}$  per cent were outside of both standards. Judging this milk from a temperature standpoint alone,  $33\frac{3}{4}$  per cent would have been condemned instead of  $21\frac{1}{2}$  per cent. Of the milk thus condemned on temperature about 72 per cent would have been within the bacterial standard, while over half of the milk which was proven unfit for use by the bacterial examination was at a temperature below  $50^{\circ}$  F. when the samples were taken.

The second year, 1905, the previous work began to show results. Inspections were made constantly, warning notices sent as before, and a few cases were fined in court for continued failure to comply with either or both regulations. The samples examined for this second year then may fairly represent a milk supply where these regulations are in effect, and an honest effort is being made to produce a milk supply complying with them. We have results for both temperature and bacterial examination of 4,453 samples for 1905 as follows: 73 per cent were within both standards; 4 per cent had high temperatures with low bacterial content; 20 per cent had high bacterial content with low temperatures, and 3 per cent were outside both standards. Judging this milk from a temperature standpoint alone, 7 per cent would have been condemned instead of 23 per cent; 60 per cent of the milk thus condemned on temperature alone would have been within the bacterial standard and 87 per cent of the milk which bacterial examination showed unfit for use would have escaped notice. These results show a gain of about 20 per cent in milk within both standards and of 26 per cent on the temperature standard alone. The smallness of the gain ( $1\frac{1}{2}$  per cent) in the amount of milk exceeding the bacterial standard may be explained by the fact that during 1905 over 1,000 samples were taken from stores and wagons, while during the first period of seven months samples were taken at the contractors' receiving stations only. Of samples taken at the receiving stations during this second year only  $12\frac{1}{4}$  per cent had a count above 500,000 to the c.c. or  $9\frac{1}{4}$  per cent less than the preceding year.

During the present year the same conditions prevail. Ice has however been very scarce, and there have been no prosecutions. Under these conditions the milk supply shows some deterioration. Nine hundred and twenty-four samples have been taken from stores

and wagons. Up to November 1, we have results for both temperature and bacteriological examination of 3,907 samples, of which  $66\frac{3}{4}$  per cent were within both standards;  $9\frac{1}{2}$  per cent had high temperatures with low bacterial content; 19 per cent had high bacterial content with low temperatures, and  $4\frac{3}{4}$  per cent were outside of both standards. Judged from the temperature standpoint alone  $14\frac{1}{4}$  per cent would have been condemned instead of  $23\frac{3}{4}$  per cent; 66 per cent of the milk thus condemned on temperature would have been within the bacterial standard, and 80 per cent of the milk which bacterial examination showed unfit for use was within the required temperature.

A summary of the samples on which both examinations were made for 29 months shows that of a total of 11,403 samples 66 per cent were within both standards;  $11\frac{1}{4}$  per cent had high temperatures with low bacterial content;  $17\frac{1}{2}$  per cent had high bacterial content with low temperatures, and  $5\frac{1}{4}$  per cent were outside both standards. Judged from the temperature standpoint alone,  $16\frac{1}{2}$  per cent would have been condemned instead of  $22\frac{3}{4}$  per cent. Of the milk thus condemned on temperature, 68 per cent would have been within the bacterial standard, while 77 per cent of the milk which was proven unfit for use from high bacterial content would have escaped notice.

More striking still is a consideration of the relation of temperature and bacterial content at different periods of the year. July is our worst summer month. We have records of 1,153 samples taken during July in the three years;  $30\frac{1}{2}$  per cent of these were within both standards;  $17\frac{1}{4}$  per cent had high temperatures with low bacterial content;  $35\frac{1}{4}$  per cent had high bacterial content with low temperatures, and 17 per cent were outside of both standards. If judged by temperature alone 37 per cent would have been condemned instead of 52 per cent. About half of the milk thus condemned on temperature would have been of low bacterial content, while over two-thirds of the milk which bacteriological examination proved unfit for use would have escaped notice. December stands at the other extreme. Of the 552 December samples on which we have records of both temperature and count,  $89\frac{1}{2}$  per cent were within both standards;  $3\frac{3}{4}$  per cent had high temperatures with low bacterial content;  $6\frac{3}{4}$  per cent had high bacterial content with low temperatures, and there were



# REGULATIONS FOR A CITY'S MILK SUPPLY

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TABLE 1.

COMPARISON OF TEMPERATURE AND COUNT ON SAMPLES TAKEN DURING 29 MONTHS.

Year	TEMPERATURE LOW COUNT LOW			TEMPERATURE HIGH COUNT LOW			TEMPERATURE LOW COUNT HIGH			TEMPERATURE HIGH COUNT HIGH		
	1904	1905	1906	1904	1905	1906	1904	1905	1906	1904	1905	1906
January.....	...	162	491	...	1	42	...	16	51	...	0	7
February.....	...	502	369	...	8	31	...	39	24	...	2	1
March.....	...	459	348	...	17	35	...	78	32	...	1	1
April.....	...	325	451	...	23	14	...	66	43	...	0	4
May.....	...	345	111	...	3	11	...	38	126	...	2	8
June.....	192	346	62	60	58	12	37	92	116	36	41	38
July.....	209	101	42	181	13	4	103	197	107	111	31	54
August.....	215	87	212	264	16	70	52	116	72	90	18	28
September.....	349	40	198	117	4	67	110	114	64	35	21	18
October.....	252	285	327	45	39	83	27	72	106	11	8	27
November.....	286	256	...	53	0	..	29	83	...	5	0	..
December.....	149	345	...	18	2	..	7	31	...	0	0	..
	1.652	3.253	2.611	738	184	369	365	892	741	288	124	186

TABLE 2.

SAME AS TABLE 1. EXCEPT THAT FIGURES FROM DIFFERENT YEARS IN THE SAME MONTH ARE ADDED.

	Temperature Low Count Low	Temperature High Count Low	Temperature Low Count High	Temperature High Count High	Totals
January.....	653	43	67	7	770
February.....	871	39	63	3	976
March.....	807	52	110	2	971
April.....	776	37	109	4	926
May.....	456	14	164	10	644
June.....	600	130	245	115	1.090
July.....	352	198	407	196	1.153
August.....	514	350	240	136	1.240
September.....	587	188	288	74	1.137
October.....	864	167	295	46	1.282
November.....	542	53	62	5	662
December.....	494	20	38	0	552
Totals.....	7,516	1,291	1,998	598	11,403

TABLE 3.

SAME AS PREVIOUS TABLE BUT EXPRESSED IN PERCENTAGE.

	Temperature Low Count Low	Temperature High Count Low	Temperature Low Count High	Temperature High Count High
January.....	84.80%	5.59%	8.70%	0.91%
February.....	80.24	4.00	6.45	0.31
March.....	83.12	5.35	11.33	0.21
April.....	83.80	4.00	11.77	0.43
May.....	70.82	2.17	25.46	1.55
June.....	55.05	11.92	22.48	10.55
July.....	30.53	17.17	35.30	17.00
August.....	41.45	28.23	19.35	10.97
September.....	51.03	16.54	25.33	6.50
October.....	67.39	13.03	16.00	3.58
November.....	81.87	8.01	9.37	0.75
December.....	89.50	3.62	6.88	0.00
Totals.....	65.91%	11.32%	17.52%	5.25%

TABLE 4.  
SHOWING COMPARISON OF TEMPERATURE AND BACTERIAL CONTENT FOR THE DIFFERENT YEARS.

	Temperature Low Count Low	Temperature High Count Low	Temperature Low Count High	Temperature High Count High	Totals
June 1 to Dec. 31, '04.....	1,652 or 54.20%	738 or 24.25%	365 or 12.00%	288 or 9.46%	3,043
Jan. 1 to Dec. 31, '05.....	3,253 or 73.05	184 or 4.13	892 or 20.03	124 or 2.79	4,453
Jan. 1 to Oct. 31, '06.....	2,611 or 66.83	369 or 9.44	741 or 18.97	186 or 4.76	3,907

no samples outside of both standards. If judged by temperature alone,  $3\frac{3}{4}$  per cent would have been condemned instead of  $6\frac{3}{4}$  per cent. All the milk thus condemned on temperature would have been good

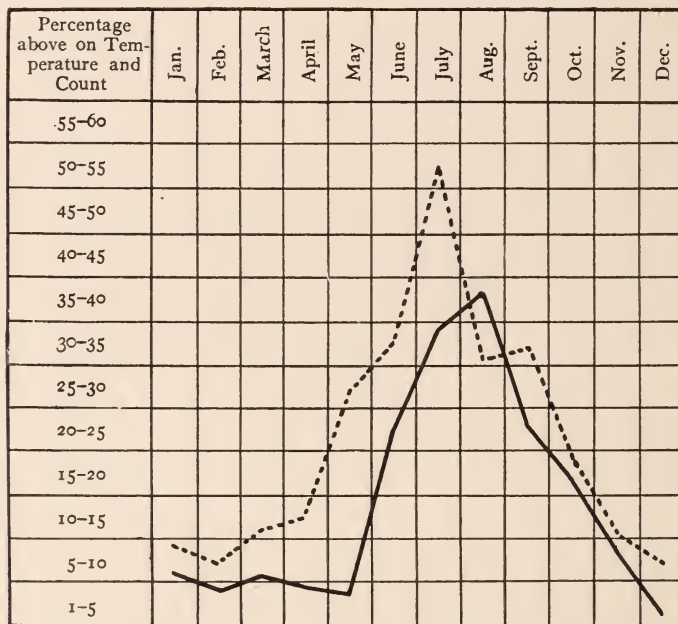


CHART 1.  
COMPARISON OF TEMPERATURE AND COUNT.

This diagram shows average high temperature (plain line) and high count (dotted line) milk for 20 months, June 1, 1904, to November 1, 1906. While they follow the same curve almost exactly, the high count milk is for the most part low temperature, and vice versa, as shown by the preceding tables. Such a chart as this is apt to be quite misleading if all the factors are not stated.

as to bacterial content, while all the milk which the bacterial examination proved unfit for use would have escaped notice.

In attempting comparison of these two tests it is necessary first to point out the relative scope of each. The dealer whose milk com-

plies with the *bacterial* standard must, we believe, have good cows, well housed and tended, must handle his milk in a cleanly manner, must cool his milk, and transmit it without delay to the consumer. These four points, emphasized many times heretofore, are the essential factors in producing good clean milk. It is evident that the temperature standard forces the observance of but one of the factors, cold, while the bacterial standard forces the observance of all four.

Unquestionably, the temperature factor is an important one in the production of good milk, especially in hot weather, since the condemnation of milk on the temperature standard alone, if widely applied at all stages of the progress of the milk from producer to consumer, would undoubtedly result in a very general observance of the temperature regulation. It can, however, never accomplish what the bacterial standard accomplishes.

The temperature test has the advantage of being less arduous, since the temperature of many samples may be taken with less labor than that required for one bacterial examination, but it is probably true that in a large city it is of comparatively little importance whether 20 or 100 samples are examined in a day, so long as samples are seized every day without notice.

If either test is to be chosen, to the entire exclusion of the other, the count is undoubtedly the one to choose, since to keep milk low in bacterial count would necessitate its being kept cold.

## COMPARISON BETWEEN BACTERIOLOGICAL ANALYSIS OF AIR BY THE PLATE METHOD AND BY FILTERS.

GEORGE A. SOPER.

Two principal methods have so far been used by investigators to determine the numbers of bacteria in air: First, Petri dishes, containing a suitable culture medium, have been exposed for the organisms to settle upon during a definite period of time; and, second, the bacteria have been collected by means of filters through which a measured volume of air has been passed.

An opportunity was afforded me during a recent investigation of the air of the New York subway to compare the serviceability of these two methods on a scale and under conditions which were capable of giving a useful idea of their relative value.

The determinations by the plate method numbered about 2,800; the determinations by the filter method, 166. The work was all done in duplicate. The air examined was the air of the New York subway and the air of the streets. As far as practicable, the observations by the two methods were made under the same circumstances as to hour and place, but they were not made simultaneously.

### THE PLATE METHOD.

The plates used were Petri dishes about  $3\frac{1}{2}$  inches in diameter. The culture-medium was beef-extract agar. The reaction, determined by preliminary experiments to ascertain the optimum, was 1 to 1.5 per cent acid to phenolphthalein.

It was customary to pour the agar into the plates at the laboratory and incubate them for twelve hours at  $37^{\circ}$  C. before exposing them to detect accidental contamination. The plates were carried from the laboratory to the point where the air was to be examined, wrapped in sterile towels, and fitted closely into a handbag. The period of exposure was 15 minutes in those cases where the numbers of bacteria were not expected to be large.

After exposure, the plates were taken to the laboratory, incubated at  $37^{\circ}$  C., and counted after 48 hours. Moulds were distinguished from the bacteria and a separate record kept of their number.



## THE FILTER METHOD.

The filter method was substantially that used by Sedgwick, Prudden, and others, with some modifications. The filters were glass tubes about 0.5 c.c. inside diameter, and 13 cm. long. The filtering material was held in place by a plug of wire gauze at one end and at the other freely exposed to the air. During transportation a plug of sterilized cotton closed the tube at each end.

After preliminary trials of various filtering materials, including some soluble ones, the medium finally adopted was sand. The depth of sand was about 5 cm. The particles were chiefly quartz. The grains ranged from about 1 mm. in diameter down to very fine particles. Two filters were always arranged in tandem.

Air was passed through the filters in most cases by means of a carefully constructed air pump. The quantity of air was determined by the number of strokes of the pump. To pump 20 liters of air through the filters, 66 strokes were required. This was the amount generally used in each case.

It was sometimes not feasible to use an air pump, where its use would attract a crowd of curious people. Under these circumstances, a brass vacuum cylinder of about 10 liters capacity, fitted with a pressure gauge and suitable stop cocks, as devised by Prudden, was employed. This cylinder was fitted into an inconspicuous leather handbag. Through suitable openings the gauge could be seen and the stop-cocks operated. Before taking a sample, the air was exhausted from the cylinder and the stop-cocks closed. The apparatus was then carried to the place where the observation was to be made. The filters were there connected to the cylinder by means of short rubber tubes and, after reading the gauge, the air was allowed to flow into the cylinder through the filters. The cocks were closed when the desired quantity of air had been filtered, as determined by a second reading of the gauge. Finally, the filters were replugged and taken to the laboratory.

At the laboratory the sand was emptied from the first filter into a test-tube which contained 10 c.c. of sterile water. After thoroughly agitating the sand and water, the organisms which were rinsed from the sand were plated in agar of similar composition to that already described as having been used in the filter method. The agar was

incubated for 48 hours at  $37^{\circ}$  C. and the colonies counted, moulds being recorded separately.

Most of the organisms were caught in the first filter. The percentage which those in the second filter bore to those in the first, as determined by 140 analyses, was 2.6.

#### RESULTS AND CONCLUSIONS.

As determined by the plate method and by the filter method, the same relation appeared to exist between the bacteria in the air of the subway and in the streets. The average numbers of bacteria which settled from the air in 15 minutes and were subsequently enumerated by the plate method were, in the subway, 500; in the streets, 1,157; ratio 1 to 2.3. The average numbers of bacteria found by the filter method were, in the subway, 3,200 per cubic meter of air; in the streets, 6,500; ratio 1 to 2.0.

This interesting relation between the results of examinations of the air of the subway and streets by the two methods should not be taken to indicate that both methods were accurate, for, as is well known, there is no precise way to determine the numbers of bacteria in air. The two methods were the most accurate which it was found feasible to employ upon the scale required. The plate method was very much more convenient than the filter method, and gave results which in this case were sufficiently instructive to warrant its almost exclusive employment as a general routine procedure. The filter method, although it probably gave a more accurate idea of the condition of the air, involved more difficulties of technique than the superiority of its results warranted. Together the two methods yielded data from which a large number of profitable deductions were drawn.

## THE AGGLUTINATION METHOD OF DIAGNOSIS IN THE CONTROL OF GLANDERS

VERANUS A. MOORE AND WALTER J. TAYLOR.

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IN the control of glanders the first and most important requirement is an accurate diagnosis. The inoculation method of Strauss gave much assistance in cases of apparent lesions, from which inoculating material could be obtained. This, however, did not afford assistance in cases of horses which had been exposed to the disease, but which did not exhibit symptoms or lesions of the malady. Mallein was welcomed as a means of detecting the disease in cases of this kind and in obscure cases generally, but its application has not been altogether satisfactory. Its limitations have not been so clearly defined as those of tuberculin, and the obstacles and complications in its use render many of the results difficult of interpretation. For these reasons a method that can be applied by a trained man, eliminating nearly if not all of the troublesome features of the mallein test, is worthy of careful consideration.

The agglutination method of diagnosis, which was first applied to glanders by M'Fadyean, was recommended in the work of control of the disease by Schütz and Miessner, and by Schnürer, and more recently studied in this laboratory, offers a possible solution for several of the difficult features in the diagnosis of glanders.

The method as formulated by Schütz and Miessner has been officially adopted for the diagnosis of glanders generally in Prussia. When there are cases of suspected glanders, the veterinarian or official in charge applies for sterile bottles, which are forwarded from the laboratory to receive the blood. The specimen of blood and the horse from which it was taken are given a number for identification, and the samples are sent to the laboratory. The test is made and the results reported, with directions for the disposition of the animal.

In Austria, Schnürer makes the diagnosis of glanders in suspected cases for the Austrian army. His method of procedure is practically the same as that of Schütz and Miessner, and he reports it as more

accurate than any other method for the diagnosis of this disease. During the last year, Moore, Taylor, and Giltner have tested the method very carefully, using it in the diagnosis of a considerable number of cases of suspected glanders with very satisfactory results. Dr. Berns, a veterinary practitioner of Brooklyn, N. Y., has used it for the last six months in his practice, and reports the most satisfactory results from it in 170 horses to which he had applied it at the time of writing.

The development of the method can be followed from the writings of M'Fadyean, Bourget and Méry, Arpàd, Fedorowsky, Rabieaux, Reinecke, and Bonome.

Schütz and Miessner recommend the use of glanders bacteria that have been killed by heating them for two hours at 60° C. The killed organisms were suspended in a carbolized-salt solution. The suspension was made of a light-greyish color and distributed in small test tubes, 2 c.c. in each. Various quantities of the diluted blood-serum were added to this emulsion, and the mixture incubated for 24-30 hours at 37° C.

The results of these experiments demonstrated that while the blood-serum of healthy horses agglutinated the bacteria in dilutions of 1-400 at the highest, the serum of glandered horses reacted in dilutions of from 1-1,000 to 1-2,000. The blood-serum of horses artificially infected with a virulent culture gave a reaction beginning on the fifth to the seventh day after inoculation, the agglutinating power increasing during the following four or five days, remaining at its maximum for about one month, and after that gradually diminishing. This is of practical importance in detecting glanders in a stable where infected horses have been destroyed, the test being made two or three weeks later. Experience led to the belief that a previous malleination had no effect on the agglutinating power of the serum.

The blood of non-glandered but diseased horses reacted occasionally in higher dilutions. Thus it is found in cases of pleurisy and pneumonia that a reaction occurred in a dilution of 1-1,000, while in other cases it did not occur in dilutions higher than 1-800. In the Pathological Institute in the Veterinary College in Berlin, during two years, the blood-serum of 2,209 horses was tested for glanders with the following results:



Of 1,911 horses free from glanders; in

1,232	or 64.8	per cent	the blood-serum agglutinated	1-100	to 1-300
363	"	19.0	" " " "	"	1-400
135	"	7.1	" " " "	"	1-500
123	"	6.4	" " " "	"	1-600
41	"	2.2	" " " "	"	1-800
11	"	0.5	" " " "	"	1-1,000

A reaction higher than 1-1,000 was not observed in a single case.

Of 298 glandered horses; in

6	or 2.0	per cent	the blood-serum agglutinated at	1-400
12	"	4.0	" " " "	" 1-500
44	"	14.8	" " " "	" 1-600
47	"	15.8	" " " "	" 1-800
75	"	25.2	" " " "	" 1-1,000
49	"	16.4	" " " "	" 1-1,500
65	"	21.8	" " " "	" 1-2,000

A study of these tables shows that the greater percentage of reactions with normal serum has been with very low dilutions, while the greater percentage with glandered serum has reacted in dilutions so high that they were positively diagnostic. Experience shows that in glandered horses the agglutinating power of the blood is with the passing of time gradually diminished, while in horses free from glanders the agglutinating power of the blood does not change. Based upon their experience, Schütz and Miessner recommend the following method for the eradication of glanders:

Twenty to 50 grams of blood are taken from the glandered or suspected horse, the date and history of the case being recorded and sent to the experiment station.

All horses whose blood agglutinates in dilutions of 1-1,000 or higher should be destroyed.

In the same way, all horses should be destroyed whose blood agglutinates in dilutions of only 1-500 to 1-800, if they show symptoms of glanders.

All other horses in which the agglutination is 1-500 to 1-800 should be isolated and destroyed only when justified by a second test, in which the maximum dilution for agglutination is changed; on the other hand, they may be pronounced free from glanders if at the second test the dilution remains unchanged.

After glanders is established, the blood of horses in the same stable should be tested after three weeks; and this should be repeated until the last two tests show in all horses individually a uniform reaction.

In our work we have applied the general method recommended by Schütz and Miessner, with such slight modifications as seemed desirable. The method for the routine diagnosis which we have found to be very satisfactory is as follows:

*Culture.*—As pointed out by Schütz and Miessner, all cultures of *B. mallei* do not agglutinate satisfactorily. It was also shown by their work that a suitable culture when obtained is liable, at unexpected intervals, to lose its responsiveness to the agglutinin. This can be prevented by passing the organisms through a guinea-pig at least once in three weeks. The organisms were grown for from 48 to 72 hours

on acid-glycerin agar (5 per cent glycerin and with a reaction of +2.9 to phenolphthalein). In order to have a suitable culture on hand, subcultures should be made daily. A culture more than 72 hours old should not be used in preparing the test fluid.

*Test-fluid.*—The test-fluid is prepared by washing the growth from the agar culture by the aid of a sterile wire loop into distilled water containing 0.85 per cent sodium chloride and 0.5 per cent carbolic-acid crystals. This suspension is then placed in a thermostat at 60° C. for two hours, which kills the bacteria. A temperature higher than 65° C. or lower than 60° C. should be avoided. After heating, the suspension is thoroughly triturated and filtered through sterile cotton. Thorough trituration of the emulsified growth is essential before filtering. The filtrate thus prepared is diluted with the carbolized-salt solution until it is of a faintly cloudy appearance. The proper dilution of the filtrate can only be determined by experience. The test-fluid gives the best results when made with freshly prepared carbolized-salt solutions.

*Procuring the serum.*—The serum is easily obtained. At least 10 c.c. of blood are drawn from the jugular vein, under aseptic precautions, into a small sterile bottle, and sent to the laboratory. As soon as the clot forms, the supernatant serum is placed in a centrifuge and all the sediment thrown down, leaving the liquid perfectly clear. One c.c. of the serum is then added to 39 c.c. of a physiological salt solution, which makes a dilution of 1-40. It is desirable that the serum should be secured as soon as possible after the blood is drawn. If necessary to delay the test, the serum has given the best results if kept at about 10° C. until used. The diluted serum tends to deteriorate if kept more than 24 to 48 hours. Even during this time it should be kept at a low temperature. Serum that has decomposed should not be used.

*Making the test.*—Three c.c. of the "test-fluid" are placed in each of several small test-tubes. With a sterile pipette, the diluted serum is added to the tubes of test-fluid and thoroughly mixed. In making the different dilutions, the amount of diluted serum to be used is readily ascertained by the following table:

TABLE 1.

Dilution of Serum	Amount of Diluted Serum	Amount of Test-Fluid	Dilution
1-40	1.2 c.c.	3 c.c.	1-100
1-40	0.6	3	1-200
1-40	0.405	3	1-300
1-40	0.3	3	1-400
1-40	0.24	3	1-500
1-40	0.195	3	1-600
1-40	0.15	3	1-800
1-40	0.12	3	1-1,000
1-40	0.105	3	1-1,200
1-40	0.09	3	1-1,500
1-40	0.06	3	1-2,000
1-40	0.03	3	1-4,000
1-40	0.015	3	1-8,000

Where dilutions greater than 1-1,000 are made, a serum diluted 1-80 may be used to better advantage, unless the pipette employed is very finely graduated. In this case the amount of diluted serum for a certain dilution must be double that indicated in the table.

The mixture thus prepared is placed in an incubator at 37° C. for 24-30 hours. A temperature higher than 37° C. interferes with the agglutination.

*Reaction.*—The reaction consists of a layer of the agglutinated bacteria covering the entire convexity at the bottom of the tube. This film-like sediment may become so dense that it rolls in at the periphery. The supernatant fluid becomes clear in the lower dilutions, but in the higher ones the clarification may not be complete, showing that all the bacteria have not become agglutinated. This is further evinced by the fact that the layer is less dense in the higher dilutions. The reaction may begin in six hours, but cannot be considered complete until 24 to 26 hours have elapsed. If no reaction appears in 24 hours it cannot be considered negative, as it may occur in from 30 to 40 hours after setting. Often, however, a reaction appears in less than 24 hours.

After the agglutination is completed, further standing produces no visible change in the test-fluid.

A negative result shows a small round concentrated spot of sediment in the center of the convexity at the bottom of the tube, the test-fluid remaining apparently unchanged even after several weeks.

In our examinations, we have confined our work very largely to the macroscopic appearances. It is believed, however, that not infrequently helpful information could be obtained by a microscopic examination as well. However, we have not found it safe to depend on a microscopic reaction for diagnosis, because of the variability of horse sera in agglutinating *B. mallei*. Often the microscope will reveal the presence of clumping, when macroscopically it cannot be detected. Our experience has led to the belief that a high dilution microscopic reaction cannot be considered diagnostic. The macroscopic precipitation of the agglutinated bacteria seems to be essential before drawing conclusions. In testing suspected blood, we have followed the plan of making for each examination dilutions of 1-200, 1-500, 1-800, 1-1,000 and 1-1,200. If a reaction took place at 1-1200 higher dilutions were tested. By this method we were able to tell if the culture were reliable by observing the reaction at the dilution of 1-200 as this should agglutinate even with non-glandered serum. If a reaction occurred in the absence of symptoms at 1-800, the case was considered suspicious and retested in from a few days to three weeks later. If a reaction appeared at 1-1,000, 1-1,200 or higher, the animal was considered glandered.

Thus far, as shown in Tables 1 and 2, we have not had a reaction with the serum from a non-glandered horse above 1-500. The majority failed to react above 1-400. In all cases where we have had a reaction of 1-1,000 or higher, the animal has shown conclusive clinical evidence of glanders, or upon postmortem examination has

exhibited characteristic lesions of that disease. This corresponds to the findings of Schütz and Miessner.

We have applied this test to the blood-serum of a total of 81 horses. Some of these were in good health; some were suffering from diseases other than glanders; still others, and by far the largest number, were believed to be glandered or suspected of having the disease because of certain symptoms; or they appeared to be healthy but had been exposed. A summary of the percentages of the maximum dilutions of the serum at which agglutination occurred is appended:

Of 19 healthy horses; in

3	or	15.8	per cent the maximum dilution was	1-200
2	"	10.5	" " " " " "	1-300
11	"	57.9	" " " " " "	1-400
3	"	15.8	" " " " " "	1-500

Of 12 diseased but not glandered horses; in

1	or	8.3	per cent the maximum dilution was	1-200
5	"	41.7	" " " " " "	1-300
4	"	33.3	" " " " " "	1-400
2	"	16.7	" " " " " "	1-500

Of 50 horses suspected of having glanders; in

1	or	2	per cent the maximum dilution was	1-3,200
1	"	2	" " " " " "	1-2,800
1	"	2	" " " " " "	1-2,000
7	"	14	" " " " " "	1-1,600
14	"	28	" " " " " "	1-1,500
12	"	24	" " " " " "	1-1,400
4	"	8	" " " " " "	1-1,200
1	"	2	" " " " " "	1-1,000
1	"	2	" " " " " "	1-800
8	"	16	" " " " " "	1-500 or less.

The dilutions in which agglutination occurred in the serum of each of 68 horses including the three classes of cases are to be found in Tables 2, 3, and 4.

The clinical history of all the cases recorded in Table 4 as reported by the veterinarians who had them in charge shows that in every case in which the macroscopic agglutination occurred in the maximum dilution of 1-500 or lower the horse did not have glanders, and that in every case in which agglutination occurred at dilutions of 1-1000 or higher the horse was suffering from some form of glanders.

The delicacy of the method is illustrated in case No. 11. The horse had been exposed, but showed at the time the blood was taken



no evidence of the disease. Three weeks later it developed numerous typical lesions. Again in No. 15 the horse exhibited suspicious symptoms, but it was thought by some veterinarians not to be glandered. After the test the horse was condemned, as it gave a positive reaction to mallein, and on postmortem was found to contain typical glanders lesions.

In two cases, not reported in this table, the agglutination occurred with a maximum dilution of 1-400 and 1-500 respectively. These we reported as negative; but the veterinarians in charge replied that the horses were glandered and had been destroyed. Unfortunately they were not examined postmortem.

It has already been pointed out by others that the agglutinating power of the serum diminishes quite rapidly after the disease becomes established. We have not had an opportunity to test this phase of the reaction.

TABLE 2.  
AGGLUTINATION OF *B. mallei* WITH SERUM FROM HEALTHY HORSES.

Number	1-100	1-200	1-300	1-400	1-500	1-600	1-700	1-800	1-900
1.....	+	+	+	+					
2.....	+	+	+	+					
3.....	+	+	+	+					
4.....	+	+	+	+					
5.....	+	+	+	+					
6.....	+	+	+	+					
7.....	+	+	+	+					
8.....	+	+	+	+					
9.....	+	+	+	+					
10.....	+	+	+	+					
11.....	+	+	+	+	+				
12.....	+	+	+	+					
13.....	+	+	+	+					
14.....	+	+	+	+					
15.....	+	+	+	+					
16.....	+	+	+	+					
17.....	+	+	+	+	+				
18.....	+	+	+	+	+				
19.....	+	+	+	+					

TABLE 3.  
AGGLUTINATION OF *B. mallei* WITH SERUM OF DISEASED BUT NOT GLANDERED HORSES.

Disease	1-100	1-200	1-300	1-400	1-500
Edema of nasal septum.....	+	+	+	+	
Acute skin eruption.....	+	+	+	+	
Azoturia.....	+	+	+		+
Azoturia.....	+	+	+		
Azoturia.....	+	+	+		
Influenza, temperature 105.3°.....	+	+	+		
Pneumonia, temperature 102.3°.....	+	+	+	+	
Typhoid pneumonia, temperature 106.3°.....	+	+	+	+	
Typhoid pneumonia, temperature 103°.....	+	+	+		+
Acute laminitis.....	+	+	+	+	
Acute influenza, temperature 106.2°.....	+	+	+	+	
Distemper.....	+	+	+		

TABLE 4.

AGGLUTINATION OF *B. mallei* WITH THE SERUM OF HORSES SUFFERING WITH OR SUSPECTED OF HAVING GLANDERS.

No.	1-200	1-300	1-400	1-500	1-600	1-800	1-1000	1-1200	1-1400	1-1500	1-1600	1-1800	1-2000	1-2200	1-2400	1-2500	1-2600	1-2700	1-2800	1-2900	1-3000	1-3100	1-3200
1.....	+	+	+	+	+	+	+	+	+	+	+												
2.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.....	+	+	+	+	+	+	+	+	+	+	+												
4.....	+	+	+	+	+	+	+	+	+	+	+												
5.....	+	+	+	+	+	+	+	+	+	+	+												
6.....	+	+	+	+	+	+	+	+	+	+	+												
7.....	+	+	+	+	+	+	+	+	+	+	+												
8.....	+	+	+	+	+	+	+	+	+	+	+												
9.....	+	+	+	+	+	+	+	+	+	+	+												
10.....	+	+	+	+	+	+	+	+	+	+	+												
11.....	+	+	+	+	+	+	+	+	+	+	+												
12.....	+	+	+	+	+	+	+	+	+	+	+												
13.....	+	+	+	+	+	+	+	+	+	+	+												
14.....	+	+	+	+	+	+	+	+	+	+	+												
15.....	+	+	+	+	+	+	+	+	+	+	+												
16.....	+	+	+	+	+	+	+	+	+	+	+												
17.....	+	+	+	+	+	+	+	+	+	+	+												
18.....	+	+	+	+	+	+	+	+	+	+	+												
19.....	+	+	+	+	+	+	+	+	+	+	+												
20.....	+	+	+	+	+	+	+	+	+	+	+												
21.....	+	+	+	+	+	+	+	+	+	+	+												
22.....	+	+	+	+	+	+	+	+	+	+	+												
23.....	+	+	+	+	+	+	+	+	+	+	+												
24.....	+	+	+	+	+	+	+	+	+	+	+												
25.....	+	+	+	+	+	+	+	+	+	+	+												
26.....	+	+	+	+	+	+	+	+	+	+	+												
27.....	+	+	+	+	+	+	+	+	+	+	+												
28.....	+	+	+	+	+	+	+	+	+	+	+												
29.....	+	+	+	+	+	+	+	+	+	+	+												
30.....	+	+	+	+	+	+	+	+	+	+	+												
31.....	+	+	+	+	+	+	+	+	+	+	+												
32.....	+	+	+	+	+	+	+	+	+	+	+												
33.....	+	+	+	+	+	+	+	+	+	+	+												
34.....	+	+	+	+	+	+	+	+	+	+	+												
35.....	+	+	+	+	+	+	+	+	+	+	+												
36.....	+	+	+	+	+	+	+	+	+	+	+												
37.....	+	+	+	+	+	+	+	+	+	+	+												

Berns and Way report the application of this method during the summer of 1906 in Dr. Berns's hospital. The following quotation from their paper is of interest:

While the agglutination test is still in its infancy and our experiments are by no means completed, and our cases not sufficient in number to warrant positive conclusions, we are of the opinion that this method is a most valuable aid in diagnosing glanders, and from the above it would seem that this test not only reveals the presence of infection, but, to a certain extent at least, the degree of infection, by the reaction being either prompt or tardy, strong or weak, and the agglutination taking place in varying proportions from 1-200 up to 1-1,200 or even higher.

The application of the serum diagnosis of glanders in state and city sanitary work appears to have many advantages over the methods heretofore employed. The blood can be drawn by any veterinarian from the suspected horse with very little trouble and sent to the laboratory. However, the fact should be kept in mind that the method

is a laboratory test. Its requirements are such that it cannot be made in any bacteriological laboratory without sufficient notice and preparation. The necessary solutions must be ready, and cultures of *B. mallei* which are readily agglutinated must be in stock and of the proper age. To keep these in hand, requires more time, labor, and expense than can be given by laboratory men for an occasional diagnosis. The practical work, therefore, will be restricted to boards of health laboratories, or those doing the sanitary work for the city or state, and perhaps to those of practitioners who have a large practice in stables where cases of glanders are of common occurrence. As in Prussia and Austria, it would seem advisable for each large city and possibly state to have at least one laboratory where this work could be done from these sterile bottles for collecting the blood, and instructions could easily be sent to any veterinarian who wished to have the diagnosis made.

#### CONCLUSIONS.

From the results we have obtained in testing the various procedures in the serum diagnosis of glanders as set forth by various investigators and summarized in the preceding pages, the following conclusions seem to be warranted:

1. The diagnosis of glanders by the agglutination method is easier and quite as accurate as by mallein. It has this advantage, that it can be used in those cases where there is a rise of temperature, and consequently where mallein could not be employed.

2. There appear to be no objections to the recommendations of Schütz and Miessner for the eradication of glanders based on this method of diagnosis.

3. The maximum dilution of normal serum that we have found capable of producing macroscopic agglutination is 1-500. This is higher than that reported by others. It occurs, however, in but very few cases.

4. The maximum agglutinating dilution of the serum of diseased horses not glandered has not exceeded that of normal serum. This is lower than that recorded by others. We recognize, however, that our experience has been quite limited.

5. The interpretation of the results where the maximum dilution

is about 1-500 gives the greatest difficulty. All cases of this kind, unless there are unquestioned diagnostic symptoms or lesions, should be retained for a subsequent test.

6. The method, while simple in its details, requires in its application the closest of attention and constant checking, because of the liability of the culture losing unexpectedly its susceptibility to the agglutinins.

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## THE GROWTH AND TOXIN PRODUCTION OF *BACILLUS DIPHTHERIAE* UPON PROTEID-FREE MEDIA.

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OF all the problems which fall within the bounds of bacteriology today, one of the most important is that which concerns the production of toxins by certain pathogenic micro-organisms. The toxin of diphtheria has, no doubt, so far as its chemical nature and conditions of formation are concerned, received the greatest amount of study from investigators. It presents, moreover, a problem in the biochemistry of bacteria, than which, to the present time, no other has yielded more satisfactory results, considered both in their relation to pure science and to their practical application in the treatment and prevention of disease. And yet there are many points in regard to the ultimate nature of these toxic products, their relation to and interaction with the antitoxins, which, to say the least, are very far from being well understood. Furthermore, it is probable that these elements of the problem must remain a matter of speculation only, until a more adequate knowledge shall have been gained regarding the chemical nature of these toxic products, and the conditions which are either favorable or necessary to their formation.

Although, in the qualitative analysis of the toxic substance produced by *B. diphtheriae*, the investigator can recognize certain organic or inorganic bodies, he is still in the dark with reference to the ultimate proteid body, whose exact constitution he wishes to determine. It is difficult to determine whether the albumoses are the toxic products, or whether the true toxin may not, in some way, be associated with them. Some investigators assume that the toxin itself may be an enzyme which has a proteolytic action and produces the virulent, but secondary, toxic products in the body of the animal. Whatever answer may be given to these diverse and often contradictory views, the fact remains that the diphtheria organism, growing upon a proteid-free medium, can synthesize a toxic substance of a proteid nature; and that the toxin is probably not (as previously claimed by Brieger

and Fraenkel) a toxalbumen resulting from the splitting up of proteid bodies within the medium.

Among the first to determine that the toxin of *B. diphtheriae* could be formed in a proteid-free medium was Uschinsky.<sup>1</sup> Since his discovery, however, reports have appeared, from time to time, stating that success has not been met with in growing the diphtheria organism upon proteid-free media. In reply to some of these criticisms, Uschinsky says:<sup>2</sup>

Ich gebe zu dass auch ich viele Misserfolge gehabt habe. Jetzt aber besitze ich eine Kultur, welche auf meine Flüssigkeit vorzüglich wächst, und weit mehr Toxin produziert, als es bei mir früher der Fall war. Selbst 1½ c.cm. einer 4-6 Wochen alten filtrierten Kultur tödten sicher ein mittleres Meerschweinchen in 40-36 Stunden mit allen für Diphtherietoxin charakteristischen Erscheinungen.

Regarding the frequency of the growth upon his medium, Uschinsky continues:

Die Ursachen meiner und anderer Misserfolge scheinen mir darin zu bestehen, dass wirklich nicht eine jede Diphtheriekultur zum Wachsen auf eiweissfreier Nahrösung geeignet ist. Junge, frisch vom Menschen genomene Kulturen sind schwer auf dieser Lösung zu kultivieren; wogegen ältere, an saprophytische Lebensweise gewöhnte Kulturen leicht wachsen.

In the experiments about to be recorded, the writer has repeated Uschinsky's experiment, and has tried certain modifications of his medium together with some entirely new combinations. In all cases it was taken for granted that toxin-formations could not occur without at least a fair appearance of growth. With this fact in mind, the first aim of the writer was to obtain the best possible growth upon proteid-free media. Although in the first set of preliminary experiments the reaction of the media was adjusted to +0.45, as recommended by Hitchens,<sup>3</sup> in the later work (including all the combinations of media herewith presented), the reaction was made slightly alkaline, as determined by litmus.

#### USCHINSKY'S MEDIUM.

The first proteid-free medium to be tried was that recommended by Uschinsky. This was made up as follows:

<sup>1</sup> Uschinsky, *Centralbl. f. Bakt.*, 1893, 14, p. 316.

<sup>2</sup> Uschinsky, *Centralbl. f. Bakt.*, 1897, 21, p. 146.

<sup>3</sup> Hitchens, *Jour. Med. Res.*, 1905, 13, p. 523.

Water . . . . .	1,000	parts
Glycerin . . . . .	30-40	"
Sodium chloride . . . . .	6	"
Di-Potassium phosphate . . . . .	2.5	"
Ammonium lactate . . . . .	6.5	"
Magnesium sulphate . . . . .	0.3	"
Calcium chloride . . . . .	0.1	"
Asparagin . . . . .	3.2	"

Upon this medium the writer attempted to grow pure cultures of the granular, the barred, and the solid-staining<sup>1</sup> varieties of *B. diphtheriae*, obtained fresh from the nose and throat of patients suffering from diphtheria in the Providence hospitals. Out of 70 pure cultures tried, only two gave a growth upon Uschinsky's proteid-free medium, after an incubation of five days or more. In these two instances, furthermore, the growth was very slight and limited to the solid-staining varieties of the organism.

It is also perhaps noteworthy that in these cases, where several tubes of proteid-free media were inoculated from the *same* blood-serum culture, usually *only one or two tubes* would manifest a growth, thus seeming to indicate that even out of a pure culture of *B. diphtheriae*, there might be only a relatively small number of individual organisms which would immediately take up growth upon Uschinsky's medium; and furthermore, that these, either by chance or by peculiar fitness, proved to be the solid-staining types of the organism.

#### NEW COMBINATIONS.

After this trial of Uschinsky's medium, the writer made use of several other combinations of proteid-free media, all of which contained asparagin for the nitrogen basis and varied somewhat, both qualitatively and quantitatively, in respect to the salt constituents. Although an immediate growth of fresh cultures of *B. diphtheriae* upon these media did not usually occur, still on some of them the growth was more frequent and slightly more profuse than upon Uschinsky's medium. Among the many new combinations tried, especially the following may be mentioned:

Asparagin . . . . .	1.50	parts
Glycerin . . . . .	2.50	"
Sodium chloride . . . . .	0.10	"
Di-Potassium phosphate . . . . .	0.10	"
Potassium nitrate . . . . .	0.03	"
Ammonium lactate . . . . .	0.08	"
Distilled water to make . . . . .	100.00	"

<sup>1</sup> Wesbrook's classification, *Trans. Assoc. Amer. Physicians*, 1900.

Upon this medium very few cultures of *B. diphtheriae* grew; in fact only two out of 20 grew at all, and these two were types of D<sup>2</sup> and E<sup>2</sup> (Wesbrook's classification).

In the following medium the percentage of glycerin and sodium chloride was increased, and calcium chloride and ammonium phosphate were added:

Asparagin . . . . .	1.00 parts
Glycerin . . . . .	4.00 "
Sodium chloride . . . . .	0.60 "
Di-Potassium phosphate . . . . .	0.03 "
Ammonium phosphate . . . . .	1.75 "
Calcium chloride . . . . .	0.01 "
Distilled water to make . . . . .	100.00 "

In this medium six cultures grew slightly, but none very well. Of these six, three were D<sup>2</sup>.

In the next medium ammonium lactate was omitted:

Asparagin . . . . .	1.00 parts
Glycerin . . . . .	4.00 "
Sodium chloride . . . . .	0.60 "
Di-Potassium phosphate . . . . .	0.30 "
Potassium nitrate . . . . .	0.03 "
Calcium chloride . . . . .	0.01 "
Ammonium phosphate . . . . .	1.00 "
Distilled water to make . . . . .	100.00 "

In this medium 14 cultures grew; one well, the others very slightly. Of the 14, eight were D<sup>2</sup>, three were granular forms, and three barred. The one that grew most luxuriantly was a D<sup>2</sup>.

Asparagin . . . . .	1.00 parts
Glycerin . . . . .	4.00 "
Di-Potassium phosphate . . . . .	0.30 "
Magnesium sulphate . . . . .	0.03 "
Potassium nitrate . . . . .	0.03 "
Calcium chloride . . . . .	0.01 "
Ammonium lactate . . . . .	0.05 "
Ammonium phosphate . . . . .	1.00 "
Distilled water to make . . . . .	100.00 "

In the above medium, which, as will be noted, contains no sodium chloride, 27 cultures grew. Of these 27, seven grew fairly well, and 19 slightly. Of these 27, moreover, 12 were D<sup>2</sup>, 6 were granular, and 9 were barred. The 2 which gave the most luxuriant growth were D<sup>2</sup>.



The fifth lot of media had the following constitution :

Asparagin . . . . .	1.00 parts
Glycerin . . . . .	4.00 "
Sodium chloride . . . . .	0.10 "
Di-Potassium phosphate . . . . .	0.30 "
Magnesium sulphate . . . . .	0.03 "
Ammonium lactate . . . . .	0.50 "
Ammonium phosphate . . . . .	1.00 "
Phosphate of iron . . . . .	0.02 "
Distilled water to make . . . . .	100.00 "

On the medium above mentioned, 18 cultures grew, most of them very slightly. Of these, 8 were D<sup>2</sup> or E,<sup>2</sup> 7 were granular, 2 were barred and 1 was a mixed culture. Owing to an accident, however, the results of growth in this series of tubes were incomplete.

In all of the above combinations of proteid-free media tried, the only source of nitrogen was either the ammonium salts or the asparagin. Generally speaking, the results in the growth or the toxin production of *B. diphtheriae* upon these media were not pronounced. In fact the growth was not sufficient to warrant the use of these media as a basis for the deeper problem of determining what definite constituents of the media favored or prevented the formation of the toxic products. But few inoculations of these first attempts at growth were made. The principal reasons for this were, that in the majority of cases the growth was too slight to even hint that there could be even the slightest toxin formation; in the second place, before the growth had progressed a suitable length of time, the rapid evaporation of the water from the tubes in the incubator so concentrated the medium that it either prevented further growth of the cultures, or produced an uncertainty as to the exact proportion of the ingredients in the solutions. It was always noticeable, however, that the forms which grew most persistently, in spite of the changes in the density of the medium, were the solid-staining varieties. Of these last, several inoculations were made, but in only one instance did the result indicate a very high degree of toxin production.

On the theory that one reason for the slight growth in the different varieties of media to which allusion has been made, might be the difficulty in assimilating the nitrogen from the ammonium compounds or from the asparagin, it was decided to make use of some other simple nitrogen-containing compounds, as glyocol or urea.

In general, the results obtained from the employment of glycol were far more encouraging than any results obtained through the use of asparagin. Urea, however, as also determined by Sullivan<sup>1</sup> in his work on the biochemistry of color-production by bacteria, was of little value, either in aiding the luxuriance of growth or for increasing the formation of toxin.

The first medium in which glycol furnished the nitrogen basis was made up as follows:

Glycerin . . . . .	3.00 parts
Sodium chloride . . . . .	0.60 "
Calcium chloride . . . . .	0.08 "
Magnesium sulphate . . . . .	0.32 "
Di-Potassium phosphate . . . . .	0.23 "
Ammonium lactate . . . . .	0.75 "
Glycol . . . . .	0.10 "
Distilled water to make . . . . .	100.00 "

Owing to the fact that several of the first few cultures with which this medium was inoculated grew without delay, a large number of cultures was not tried. The first two cultures which grew well had been on blood serum for a period of three weeks and were transferred to the proteid-free medium from a broth culture. Of these two, one was a culture of CC<sup>1</sup>, the other of CC<sup>2</sup>. Of a 16-day-old culture of CC<sup>2</sup> in the medium specified above, 5 c.cm. were inoculated into a guinea-pig weighing 533 grams. The pig died in 36 to 38 hours with the characteristic signs of diphtherial poisoning. The organism was recovered at the autopsy in the form of a D<sup>2</sup> culture.

In still another case, a culture of CC<sup>2</sup>, having been grown for a period of 17 days in media of the same composition as the foregoing, killed a pig in 31 hours with typical diphtherial poisoning. In this case also, the organism was recovered in the form of a culture of C<sup>2</sup>D<sup>2</sup>. These results appear to indicate that glycol, even in so small an amount as 0.1 per cent, is able to render possible, provided the other constituents of the medium are favorable, a good growth and a powerful toxin production of *B. diphtheriae* upon proteid-free media, even if the period of growth is no longer than 16 days. We recall that Uschinsky states that the period of growth of his cultures was from four to six weeks.

<sup>1</sup> Sullivan, *Jour. Med. Res.*, 1905, 14, p. 109.



TABLE 1.—Continued.

	5			6			7			8		
Am. Lactate.....	0.5			0.75			3.2			3.25		
Am. Phosphate.....	1.0											
Asparagin.....	1.0											
Cal. chloride.....							0.05			0.04		
Ferric phosphate.....	0.02			0.08								
Glycerin.....	4.0			3.4			15.0			17.0		
Glyocol.....				0.1						0.5		
Magnesium sulphate.....	0.03			0.32			0.15			0.16		
Potas. nitrate.....												
Di-Potas. phosphate.....	0.3			0.23			1.25			1.25		
Sodium chloride.....	0.1			0.6			3.0			3.0		
Urea.....							1.6			2.5		
	CD	C'D <sup>1</sup>	C'D <sup>2</sup>	CD	C'D <sup>1</sup>	C'D <sup>2</sup>	CD	C'D <sup>1</sup>	C'D <sup>2</sup>	CD	C'D <sup>1</sup>	C'D <sup>2</sup>
Growth.....	S	S	S	L	L	L		S	L	S	S	L
Toxin production.....				H	H					M	M	

EXPLANATION.—In every case except Nos. 7 and 8 water was added to make 100 parts; in these to make 500 parts. S=slight, G=good, L=luxuriant growth; H=Inoculation resulted in death in less than forty-eight hours; M=Inoculation resulted in death in more than forty-eight hours.

It may be stated here that the three last-mentioned combinations of proteid-free medium were also tried, made up with agar in solid form. Although many other forms of bacteria developed luxuriantly upon these media, the growth of *B. diphtheriae* was slight. It was, however, in several instances sufficiently rapid to enable the writer to make a diagnosis of the diphtheria organisms after a period of 12 hours' growth.

#### STUDY OF ADAPTATION.

One other point of incidental interest was to determine the possibility of adapting to a proteid-free medium cultures of *B. diphtheriae* which, when fresh from the throat of man, would not grow at all on the proteid-free combinations. It is a well-known fact that many plants may, through a long and gradual process of adaptation, be fitted to live and grow in a new and materially different environment. In the present case the adaptation process was carried on as follows: To one part of broth were added portions of the proteid-free medium, in the following parts: 1, 2, 4, 6, 8, 10, 14, 18, 25, 30, 40, 50, 60, 80, 100, 140, 180, 210, 240, until finally the proteid-free medium was reached in its purity. In this system of adaptation, the tubes were inoculated successively, each from the tube preceding. A period of from 24 to 36 hours was allowed for the growth in each of the first tubes of the series, while for the tubes in the last of the series a longer period of time was found to be necessary.



By this process of adaptation five cultures of *B. diphtheriae*, of which two were of the D<sup>2</sup> type and the other three the CC<sup>+</sup> type, were brought successfully to the eighteenth series of adaptation tubes, in which the medium contained one part of broth to 210 parts of proteid-free medium. In every instance the solid-staining forms grew the most luxuriantly. In every instance, moreover, except in one culture of D<sup>2</sup>, a strong toxin was developed, and the degree of toxicity appeared to be directly proportional to the age of the culture. After the cultures had once taken a hold, so to speak, any increment in the proteid-free basis appeared to make little difference in the strength of the toxin produced in the tubes beyond the fourth series (i. e., broth 1 part, proteid-free medium 6 parts). The records of the inoculations of the barred and the granular forms during the process of adaptation may be tabulated as follows:

TABLE 2.

Series	Age of Culture	Composition	Killed in
1.....	3 weeks	Broth 1 pt.; Prot.-free 6 pts.	25 hours
2.....	2 weeks	Broth 1 pt.; Prot.-free 80 pts.	32 hours
3.....	3 weeks	Broth 1 pt.; Prot.-free 210 pts.	28 hours

These results seemed sufficiently definite to warrant the statement that certain forms of the diphtheria organism which will not grow normally upon a proteid-free medium can, by slow degrees, be fitted to such a life, and may be made to produce under such new life conditions a very strong toxin. There still remains a problem of interest to be solved in determining whether the adaptation results from a modification of the existing forms, or whether a selection of those varieties which are most suited to the new environment takes place. Regarding this question there are no data to be brought forward at this time.

#### PERIOD NECESSARY TO OBTAIN MAXIMUM TOXICITY.

There is a general unity in the opinion that the maximum toxicity for *B. diphtheriae* growing in broth is reached in from 40 to 50 hours after the inoculation. Uschinsky, on the other hand, ascertained that a period of from four to six weeks was required for the formation of a powerful toxin in his proteid-free media. In the tests which the

writer made in this regard, it was quite apparent that the food materials in a proteid-free medium were much more slowly assimilated than in the case of a broth culture. In the majority of cases at least a day was necessary before the growth was at all noticeable; and the growth was seldom heavy before three to five days had elapsed. A number of instances appeared to indicate that the degree of toxicity, at least within certain limits, was directly proportional to the time that the culture had been in incubation.

RELATION OF THE DEGREE OF TOXICITY TO THE FORM OF THE ORGANISM AND TO THE LUXURIANCE OF GROWTH.

There can be but little doubt that the granular forms, so called, of *B. diphtheriae* are, as a rule, the most virulent. The solid-staining varieties, on the other hand, appear to be non-pathogenic in the greater number of cases. And yet the results of the writer's observations permit of little doubt that a single morphological variety of the diphtheria organism is decidedly modifiable; and that not only may the granular types of the organism be resolved into the solid-staining forms, but that the opposite may also be true. Furthermore, it is clear that the solid-staining types may, either with a maintenance of their original form, or with a change of that form within the body of the animal, prove highly pathogenic for guinea-pigs. The observations in this regard, were only incidental to the main problem in hand; but, in view of the prevalent difference of opinion upon this question, they may perhaps be appropriately mentioned at this time. On the first trial, eleven inoculations of pure cultures of D<sup>2</sup> were given to guinea-pigs before one culture proved to be pathogenic. The twelfth, a D<sup>2</sup> type, killed in 39 hours, with all the usual signs of diphtherial poisoning. When the organism was again recovered, it was found to be of the barred variety. On several other occasions also, when granular forms were inoculated, barred and solid-staining forms were recovered. In three successive cases where two cultures of CC<sup>1</sup> and one culture of C<sup>1</sup> C<sup>2</sup>, grown for 17 days upon proteid-free media, were inoculated into guinea-pigs, they killed in from 37 to 59 hours, with all the characteristic symptoms. Without an exception, when the organism was recovered from the bodies, the D<sup>2</sup> form was alone present. One of these recovered cultures of D<sup>2</sup>, after 48 hours of incubation in broth, was inoculated into a guinea-pig and resulted in

death in 37 hours. The organism was recovered in the form of a D<sup>2</sup>.

These facts appear to indicate beyond a doubt that important changes may be brought about in the form and toxin production of individual bacilli in a single culture of the organism; and that these different forms are determined in a great measure by the nature of the environment. Whether this distinct modification occurs as a result of a morphological change in all the individuals during a few successive generations, or whether by a less rapid process of selection and elimination of those morphological varieties, or different strains of the same morphological variety, which are not readily adaptable, this we are not yet able to determine. It is not altogether improbable, however, that there may be in the life of the diphtheria organism what may be roughly called a series of adaptive forms, each one of which may be best suited to a circumscribed condition of environment, there to produce, as the case may be, color, toxin, or even definite changes in form.

#### TOXICITY AND LUXURIANCE OF GROWTH.

As to the relation between the degree of toxicity and the luxuriance of growth, the experience of the writer would not warrant the drawing of any far-reaching conclusions. It is true, however, that the solid-staining types, whether they be true *B. diphtheriae*, pathogenic pseudo-diphtheria bacilli, or non-pathogenic pseudo-diphtheria bacilli grow more rapidly upon proteid-free media than do the granular types. Not only do the D<sup>2</sup> forms grow more rapidly, but the nature of their growth is far more luxuriant and of quite a different nature from that of the granular varieties. The typical growth for the granular type, on either broth or proteid-free media, is at the surface, where it forms a more or less delicate film. Upon shaking the tube, this film falls to the bottom as a fine precipitate or sediment. The medium from the surface to the bottom of the tube is, in the growth of the granular forms, nearly as clear as the contents of a sterile tube. It is the nature of the solid-staining types, on the other hand, instead of forming the surface growth and maintaining a clear mid-liquid, to produce in the medium a uniform cloudiness. This may, in time, settle to the bottom of the tube, but the medium never wholly loses its turbidity. This condition of growth was observable in all the

series of cultures in proteid-free media as well as in the latter part of the series of adaptation tubes which have been mentioned above. In summation it may be said that, although a high degree of toxicity may often accompany a luxuriant growth, luxuriance of growth can never be used as a criterion of the degree of toxicity.

It may be of interest to mention one other point regarding the so-called involution-forms of *B. diphtheriae*. These, known by Wesbrook's classification as the "A" varieties, are generally conceded to represent forms which have become attenuated, both in virulence and in luxuriance of growth. This is indicated by the fact that when a culture of *B. diphtheriae* is grown upon a medium unfavorable to its growth, many of these forms develop, and they are often common in the throats of those who are recovering from an attack of diphtheria. In the writer's experience, these forms often developed, at the very last, in tubes of proteid-free media wherein the granular, and less frequently the solid-staining types, had found growth most difficult. These same involution-forms, in at least three cases, in our experiments, produced such a rapid and luxuriant growth in broth, that we were deceived by their macroscopic appearance into believing that these particular tubes were contaminated with some one of the large, pellicle-forming spore-bearers. By the examination of one tube, however, it was found that there was naught in the tube but a pure culture of the "A" varieties of *B. diphtheriae*, and that these composed, not only the heavy pellicle, which, in 24 hours, had become so dense that it restrained the liquid portion when the tube was inverted, but also the rapidly growing individuals beneath the pellicle. These individuals were the largest that have ever come under our observation, many of them measuring nearly ten micra in length, and under  $\frac{1}{16}$ -inch oil-immersion, rendering camera lucida drawings easily possible. It may also be noted that two of these cultures, referred to above, came originally from monstrous colonies growing upon blood-serum, one of which was 10 mm. in diameter. These were the only colonies of this nature ever seen by the writer.

#### SUMMARY.

1. *B. diphtheriae* will grow readily and luxuriantly in proteid-free media, and will produce therein as strong a toxin as in ordinary broth, though much more slowly.



2. Of the three nitrogen bases tried (asparagin, urea, and glycol), urea seemed to be of slight value, while glycol furnished the best growth and the strongest toxin. Asparagin appeared to give better results than urea, though it was not as satisfactory as was glycol. There is no added value to be gained by using any two, or even three, of these compounds together.

3. Upon the following medium, cultures of *B. diphtheriae* were grown, and, after an incubation of 16 days, killed guinea-pigs in the course of 36 to 38 hours:

Glycerin . . . . .	3.40 parts
Sodium chloride . . . . .	0.60 "
Calcium chloride . . . . .	0.08 "
Magnesium sulphate . . . . .	0.32 "
Di-Potassium phosphate . . . . .	0.23 "
Ammonium lactate . . . . .	0.75 "
Glycol . . . . .	0.10 "
Distilled water to make . . . . .	100.00 "

4. In several instances there were obtained cultures of pathogenic, solid-staining varieties of *B. diphtheriae* (C<sup>2</sup>, D<sup>2</sup>, E<sup>2</sup>), which, upon inoculation, killed guinea-pigs in 37 to 60 hours.

5. Cultures of *B. diphtheriae* which will not at first grow upon proteid-free media, may be adapted to it by slow degrees; and whether during the process of adaptation, or after the adaptation, it is the solid-staining forms of the diphtheria organism which always manifest the most rapid and luxuriant growth.

6. A single morphological variety of *B. diphtheriae* is decidedly modifiable, and there are indications that there may be in the life of the diphtheria organism what may be roughly called a series of adaptive forms, each one of which is best suited to a certain condition of environment, where it may produce color, toxin, or change in form.



## THE LIBERATION OF FORMALDEHYDE THROUGH THE AGENCY OF CALCIUM CARBIDE.

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DURING the last two years a very considerable amount of work has been done at the Maine Laboratory of Hygiene upon the matter of formaldehyde disinfection. In the course of this work the formalin-permanganate method was first worked out as a means of practical disinfection. In one way this method seemed an anomaly, in that part of the agent which was to do the work of disinfection was itself used up in the reaction that furnished the heat to evaporate the formaldehyde solution. In looking for a means of surmounting this difficulty, the use of calcium carbide was suggested, and the subsequent work with this compound forms the basis of this paper.

As is well known, when calcium carbide and water are mixed, there results a violent reaction from which acetylene gas is formed and driven off, while calcium oxide is theoretically the product remaining in the generator. In actual practice the residue in the generator is calcium hydroxide, resulting from the union of the first-formed oxide with water. The value of this reaction for disinfection does not lie in the products formed, but in the heat which results from the reaction. It was purposed to use this heat to evaporate the formaldehyde that was to be added to the calcium carbide, just as in the formalin-permanganate method the heat resulting from the oxidation of a portion of the formaldehyde was employed to evaporate the unoxidized portion. In this case it seemed probable that there would not be the destruction of formaldehyde noted in the other method.

In the work ordinary commercial calcium carbide and formaldehyde solution were used. The particular lot of formaldehyde solution used assayed 35.27 per cent by weight of formaldehyde. These reagents were used, as it was desired to get only such results as would be obtained in actual disinfection by this method, if it turned out to be of any value.

The first work necessary was to determine the proportions of the reagents to be used. In this work it was first noted that when the

formaldehyde was added to the calcium carbide, the formaldehyde being in its original strength, there was practically no reaction. In other words there did not seem to be enough water to react with carbide in the formalin solution alone. By using enough carbide to leave a thoroughly dry residue in the generator it was found that the formalin solution should be diluted by its own volume of water in order to give the best results. Starting then with a solution half water and half formalin, a large number of tests were made to determine the amount of carbide necessary to give a dry residue in the generator and at the same time leaving as little as possible of the carbide unused. It was finally decided to use six grams of the carbide to every 10 c.c. of the diluted formaldehyde solution. Experiments in using the large amounts of the reagents in the disinfecting-room gave as satisfactory results with these proportions as did the smaller amounts in preliminary experiments.

The work done in determining the proper proportions of the reagents showed that the best results were to be obtained when the carbide was in size about that of a pea. If in a powdered form the reaction was too violent, and if much larger than a pea the reaction was so slow as to produce an insufficient amount of heat to evaporate the formaldehyde. At the same time it was noted that a rather low dish, with walls about eight or ten inches high, and with a very wide bottom was preferable for a generator. The reagents and generator being as above described, the reaction, which starts rather slowly, is over in between six and seven minutes.

To determine the amount of formaldehyde liberated by this reaction, air was drawn from the room in which disinfection was going on. The apparatus used for this purpose was the one formerly used in determining the formaldehyde in rooms charged by the formalin-permanganate method, fully described in the paper on that work in the *Fourteenth Report of the Maine Board of Health*. In brief the apparatus was this:

A glass tube was passed through a hole in the door of the disinfecting-room so as to project about three feet into the room, the end in the disinfecting-room terminating in a funnel. The end of this glass tube, which was outside the disinfecting-room, was connected to the first of a series of three Dreschsel gas bottles, each containing 75 c.c. of distilled water. The last of the series of Dreschsel bottles was connected with a 10-liter bottle, filled to the 10-liter mark with water, any amount of which

could be siphoned off at pleasure. All rubber connections were sealed with paraffin wax and thoroughly tested to see that they were air-tight. The stoppers of the several bottles were also sealed in with paraffin wax.

In practice 20 minutes were allowed to elapse after the reaction was over before the siphon was started, so that there might be time for the gas to diffuse uniformly throughout the room. The apparatus was then allowed to run at such a rate that at least 35 minutes were required to draw 10 liters of air through the Dreschsel bottles. The pressure conditions were then equalized and the gas bottles were disconnected, their contents were poured into a 500 c.c. flask, each bottle washed three times with distilled water, and the washings added to the contents of the 500 c.c. flask. To the contents of the flask were now added 10 c.c. of a solution of standard potassium cyanide; the flask was well shaken, allowed to stand for five minutes, then 10 c.c. of a solution of N-10 silver nitrate, previously acidified with 10 drops of 75 per cent c.p. nitric acid, added; the whole made up to 500 c.c.; mixed; 100 c.c. filtered, and the excess of silver in the filtrate determined by Volhard's method. From this the amount of formaldehyde in the 10 liters of air examined was calculated, and, knowing the cubic contents of the room and the amount of formaldehyde introduced, the percentage of the original amount of formaldehyde that was yielded by the reaction was easily calculated. On three different tests the amount of formaldehyde found in the room ranged from 6.12 per cent to 11.4 per cent.

When the room was opened at the end of four hours the odor of formaldehyde was not so strong as to cause any annoyance to either the eyes or to the throat, even when the formaldehyde was used in the proportions of 500 c.c. to 1,000 cubic feet. In fact there was not so much trouble to be experienced in entering the room thus charged as there was in entering the same room when charged by the formalinpermanganate method with 200 c.c. to 1,000 cubic feet. Neither was the odor of acetylene very strong. It cannot be expected that all of this loss of formaldehyde is to be accounted for by leakage, as this did not happen when simply formaldehyde was evaporated into the room. It is possible that the mixture of the two gases—acetylene and formaldehyde—may diffuse more rapidly than formaldehyde alone, as a stronger odor of acetylene was always to be noted outside of the disinfecting-room than was ever the odor of formaldehyde, when this alone was used in charging.

Just before the completion of the reaction, when the effervescence was at its height, a yellowish-brown scum began to form over the entire surface of the liquid, and this rapidly thickened, so that in a short time the gas seemed to have difficulty in forcing its way through the scum. This scum seemed to increase in amount from the time of its appearance until the end of the reaction. No chemical examination was made of this scum, as the object of the work was to deter-

mine the bacterial efficiency of the method rather than to determine the chemical products formed. It is possible that the aldehyde, in the presence of the hot alkali, formed some solid condensation product, as aldehydes often do under these conditions. This would of course cause a considerable loss of formaldehyde for disinfecting purposes. At any rate the yield of formaldehyde by this process is very low, and the bacterial results reflect this condition.

The bacterial work was done in a room having a capacity of 862 cubic feet, measuring  $20 \times 4 \frac{1}{2} \times 10$  feet high, and containing a small jog at one end. The room had one door measuring  $3 \times 7$  feet, and two windows. One was on the outside of the building, measuring  $3 \frac{1}{4} \times 6 \frac{1}{4}$  feet, and the other opened into another room. This window was 8 feet from the floor, and measured  $1 \times 9 \frac{1}{2}$  feet. No attempts were made to make the door or windows air-tight, they being left in their natural condition so as to have disinfecting conditions as near those actually encountered by the health officer. The interior of this room was papered. The technique of the bacterial work was as follows:

A piece of heavy glazed paper was bent so as to form a shelf. Then five strips of filter paper were inoculated by loops of broth or heavy smears of blood-serum cultures of the organisms to be used. Each strip was picked up with sterile forceps, and, with sterile shears, cut into two pieces. One piece was put on the shelf of glazed paper, and the other dropped into a tube of bouillon as a control. Five strips, inoculated with the same organism, were put on each paper shelf, and the latter then placed in the disinfecting-room. Here the paper shelves were distributed in all parts of the room, and also at all heights from the floor to the ceiling. When the requisite number of cultures were in position the reagents were mixed and the room closed for four hours. At the end of this time each paper shelf was removed from the room; the pieces of infected filter-paper picked up with sterile forceps; introduced into separate tubes of sterile bouillon, and incubated at  $37.5^{\circ}\text{C}$ . for 196 hours, at the end of which time they were examined. All growths were examined microscopically to see if they were those put upon the paper or were the result of contamination of some sort.

In the work with the formalin-permanganate method the proportions of 200 c.c. to 1,000 cubic feet gave very satisfactory results and work was started using these proportions. The cultures used were colon, diphtheria, pneumococci, pyocyaneus, albus, tetragenus, typhoid, prodigiosus, and mixed cultures from throat swabbings. The whole number of cultures exposed here was 335. The work was done on three separate days, the temperatures being respectively  $73^{\circ}$ ,  $74^{\circ}$ ,  $74^{\circ}\text{F}$ . The average increase in the humidity in the test-room was 11.3 per cent.



TABLE 1.  
DISINFECTION TEST, 200 C.C. TO 1,000 CUBIC FEET.

Culture	Number	Growth	No Growth
Colon.....	35	1	34
Diphtheria.....	30	7	23
Pneumococci.....	35	4	31
Pyocyaneus.....	40	20	20
Albus.....	45	13	32
Tetragenus.....	30	2	28
Typhoid.....	25	2	23
Prodigiousus.....	30	0	21
"Mixed".....	65	20	45
Total.....	335	78	257

These proportions not giving satisfactory results the amount of formaldehyde was raised to 300 c.c. to 1,000 cubic feet. Here again work was spread over three separate days, their respective temperatures being 79°, 81°, and 70° F., and the average rise in humidity in the test-room was 11.8 per cent. Three hundred and forty-nine cultures were exposed; 168 of these were buried cultures. The results follow.

TABLE 2.  
DISINFECTION TEST, 200 C.C. TO 1,000 CUBIC FEET.

Culture	Number	Growth	No Growth
Pneumococci.....	25	2	23
Pyocyaneus.....	48	4	44
Albus.....	53	0	53
Streptococci.....	9	0	9
Typhoid.....	48	4	44
Prodigiousus.....	23	2	21
Colon.....	25	1	24
Tetragenus.....	16	2	14
Diphtheria.....	31	19	12
Aureus.....	20	19	1
Throat.....	26	2	24
"Mixed".....	25	19	6
Total.....	349	74	275

The results being again unsatisfactory the proportions were raised to 500 c.c. to 1,000 cubic feet. The work was done on three separate days, the temperatures being 74°, 79°, and 76° F. The average increase in the humidity was 13.2 per cent. In all, 268 cultures were exposed of which 120 were buried and 148 were open cultures. The method of burying the cultures was that used in my work on the formalin-permanganate method, and there fully described. In brief it was as follows:

A piece of tin six inches square had a circular hole cut in the center. Over this were spread the requisite number of thicknesses of whatever kind of cloth was used—



silk, cotton-flannel, or ticking. On the upper layer of the cloth were placed five inoculated slips of filter-paper, so as to be over the circular hole. On top of these was spread the same number of cloths as were below, the whole being capped by duplicate of the tin bottom, and then the edges were clamped tight so that the only way for the gas to reach the organisms was by penetrating the layers of cloth exposed by the holes in the tin.

TABLE 3.  
DISINFECTION TEST, 500 C.C. TO 1,000 CUBIC FEET.

Culture	Number	Growth	No Growth
Colon.....	30	0	30
Diphtheria.....	40	0	40
Pneumococci.....	30	0	30
Pyocyanus.....	20	0	20
Albus.....	13	0	13
Typhoid.....	63	0	63
Subtilis.....	22	5	17
Anthrax.....	30	0	30
"Mixed".....	20	0	20
Total.....	268	5	263

From these results it seems that it is possible to get efficient disinfection by this method provided that use is made of not less than 500 c.c. of formaldehyde solution for every 1,000 cubic feet of room space to be disinfected. The method does not compare favorably with the formalin-permanganate method, it requiring twice the amount of formaldehyde for efficient disinfection that the latter does. With the proportions used by me there was not such an increase in the humidity of the test-room as there was with the permanganate method. It may be that under some conditions this method may be of use. The acetylene and formaldehyde gases had no more effect on flies and mosquitoes than does formaldehyde gas alone.

TABLE 4.  
SUMMARY

	Growth	No Growth	Total
500 C.C.....	5	263	268
300 C.C.....	74	275	349
200 C.C.....	78	257	335
Total.....	157	795	952

## THE LIBERATION OF FORMALDEHYDE GAS FROM SOLUTION BY MEANS OF POTASSIUM PERMANGANATE.

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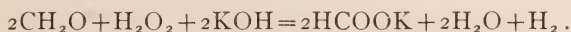
SEVERAL years ago, the writer, while studying the action of formaldehyde on camphoroxime, accidentally brought some methyl alcoholic solution of formaldehyde in contact with powdered potassium permanganate. The result was a violent explosion. A short time later the experiment was repeated, using methyl and ethyl alcoholic solutions of formaldehyde and also with the common 40 per cent aqueous solution. The results were not at all as expected. Instead of a violent explosion, a vigorous reaction took place, producing a large amount of heat and liberating formaldehyde gas.

At the time these experiments were made, formaldehyde was not used extensively for disinfecting purposes, so that the chief interest in the reaction centered about the amount of heat produced. A great number of calorimetric determinations were made. The results, however, were not entirely satisfactory. Variable results were obtained, although experiments were exactly duplicated. Early in these calorimetric determinations it was observed that when the formaldehyde was slowly dropped on powdered potassium permanganate, as each drop came in contact with the permanganate a little tongue of fire was produced and the amount of carbon dioxide was increased. These calorimetric determinations were suddenly stopped, owing to the fact that a second explosion occurred, badly injuring the calorimeter.<sup>1</sup>

In the study of the heat of combustion, a number of difficult problems had to be solved before satisfactory calculations could be made. First, the amount of formaldehyde liberated had to be determined as well as the polymerized formaldehyde left in the permanganate residue;

<sup>1</sup> A third explosion occurred some years later, while Mr. Rodney West was continuing these heat determinations. In this case the force was so violent as to render him unconscious, and these heat determinations were again discontinued.

and second, the amount of carbondioxide and formic acid had to be determined. In the determination of formaldehyde, the various methods were tried and were not found satisfactory, especially in the determination of the paraformaldehyde left in the residue. A new method was therefore worked out whereby the aldehyde in either form could be determined accurately and without great difficulty.<sup>1</sup> The above method depends upon the fact that when formaldehyde is brought in contact with hydrogen peroxide in the presence of an alkali, pure hydrogen is liberated according to the following reaction:



This method was carefully compared with the other methods and used in all the determinations which follow. It is believed that this method for determining formaldehyde will supplant the titration methods, as nothing but potassium hydroxide and hydrogen peroxide and a graduated tube are necessary for the determination.

In these determinations of formaldehyde gas liberated by potassium permanganate, it was found that the conditions under which the substances are brought together would vary the amount of formaldehyde gas. The condition of the permanganate, whether in crystal form or whether coarsely or finely powdered, made a great difference in the reaction. The strength of the formaldehyde solution and the rate of flow upon the permanganate likewise make a great difference both in the amount of formaldehyde gas liberated and in the amount of heat produced. In fact, so many variables enter into the reaction, that the calorimetric determinations were laid aside for a time and the conditions under which the greatest amount of formaldehyde gas could be liberated by permanganate were studied.

#### DETERMINATION OF FORMALDEHYDE GAS.

Several obstacles were encountered in the determination of the amount of formaldehyde gas liberated from its solution by potassium permanganate. One of the first difficulties was the retention of the gas, so that it could be accurately determined, the evolution of gas being so violent that the ordinary gas-bag stop-cock is not sufficiently large to allow the gas to escape without producing an enormous pressure upon the generator. Again, in the case of absorption of the gas,

<sup>1</sup> See "Gasometric Determination of Formaldehyde," *Jour. Amer. Chem. Soc.*, 27, p. 714.

it is extremely difficult to retain all of the gas either by water or by ammonia.

Another difficulty was experienced in bringing the formaldehyde solution in contact with the permanganate. If aldehyde is allowed to drop on permanganate from a burette, the permanganate which comes in contact with the aldehyde is changed to the oxides of manganese which seem almost impervious to the aldehyde solution, thus preventing further action. If, on the other hand, the formaldehyde is added all at once, the gas is liberated almost with explosive violence, but the amount of gas liberated is considerably less than when the solution is poured slowly over the permanganate.

An attempt to overcome this violent reaction was made by diluting the formaldehyde solution with water. It was found, however, that while the addition of water modified the reaction, the quantity of formaldehyde gas liberated was greatly diminished. Finally, the dilution of the permanganate by some inert substance was tried and found to give satisfactory results. Potassium permanganate was powdered and mixed with clean sand and the formaldehyde slowly added to this mixture. The sand prevented the formation of the impervious coating of oxides of manganese. The solution percolated through the mixture and the reaction continued as long as the aldehyde was added, or as long as there was unchanged permanganate left in the generator. As the gas is liberated from the solution by the heat of the reaction and as the specific heat of the sand is much less than that of water, more heat and hence more gas must necessarily be liberated by diluting the permanganate with sand than by diluting the formaldehyde solution with water. After many trials, it was found that a mixture of equal parts of sand and permanganate gave best results. With this proportion the reaction was violent, but not to such an extent as to make the collection of the gas impossible.

Several forms of apparatus were tried. That indicated in Fig. 1 was adopted as giving satisfaction, whether the gas was collected in gas bags and measured, or determined by absorption.

A wide-mouth, short-neck flask *A*, of about 200 c.c. was fitted with a double-bored rubber stopper. Through one hole passed a burette for introducing a known quantity of formaldehyde. Through the other passed a delivery tube *E* of large diameter. This delivery tube was connected with an aspirator bottle *B*, filled with water, one liter giving best results. The side neck of the aspirator bottle was con-

nected with an absorption tube *F*. The absorption tube was so placed that the upper end was below the surface of the water in *B*, thus slightly diminishing the atmospheric pressure upon the gas. The other end of the absorption tube was connected with another aspirator bottle *C*, which serves as a receiver in case the gas is not entirely absorbed as soon as it passes into *B*. If the reaction is very violent, forcing all of the water out of *B* into *C* by rapid liberation of gas, loss will be prevented by bubbling through the water in the absorption tube. At the end of the reaction the water is finally drawn back into *B*, but is prevented from being drawn over into *A* by the air which filled the generator at the beginning of the experiment. Usually, the absorption tube is unnecessary except as a connecting tube between *B* and *C*. When all the gaseous formaldehyde has been absorbed by the water, it is thoroughly mixed, measured, and the formaldehyde determined. During the reaction the temperature in the generator is practically constant at the boiling-point of water.

In the following table of analyses the solution used was 37.8 per cent of formaldehyde and the permanganate used was mixed with an equal amount of sand. The quantity of water used in absorbing the gas was varied, but 500 to 1,000 c.c. seemed to give the best results when 50 c.c. of the formaldehyde solution were used. With very large quantities of water the solution became so dilute as to make an exact determination of formaldehyde somewhat difficult.

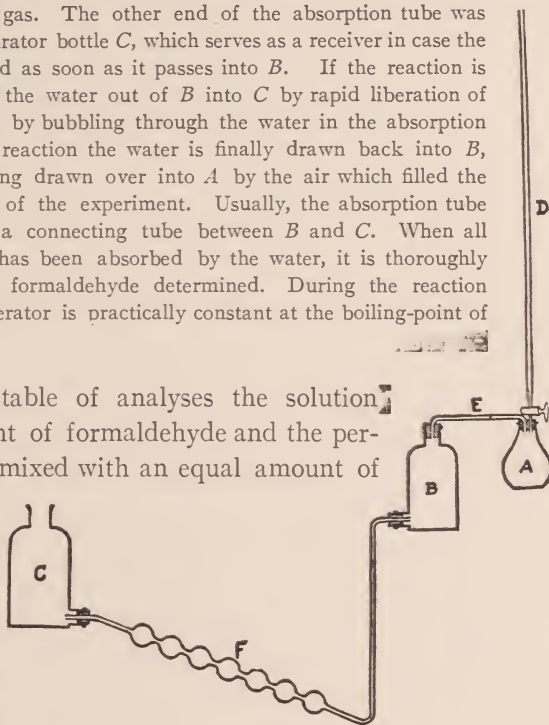


FIG. 1.

TABLE 1.

	KMnO <sub>4</sub> + Sand Grams	Formaldehyde Solution.	Volume of Water Used in Absorption	CH <sub>2</sub> O Liberated Per Cent
1.....	100	50 c.c.	1,000 c.c.	69.12
2.....	100	50	1,000	71.42
3.....	150	50	1,000	65.92
4.....	150	50	1,000	63.53
5.....	200	50	1,000	71.77
6.....	200	50	1,000	70.00
7.....	200	50	500	64.53
8.....	200	50	500	64.53

A large number of determinations were made, varying the time of reaction or the rate of flow of formaldehyde solution on the perman-



ganate. The following table gives the average percentage of gas liberated:

TABLE 2.

Number of Analyses	Time of Reaction	CH <sub>2</sub> O
	Minutes	Per Cent
3.....	80	65.06
3.....	60	68.06
6.....	45	73.11
3.....	30	74.56
2.....	15	73.56
9.....	10	70.48

In these determinations 200 grams of the mixture of sand and permanganate and 50 c.c. of 38 per cent formaldehyde were used. During the process a considerable quantity of water distilled over. The greater part of the water came from the formaldehyde solution; a small quantity was formed by the oxidation of the formaldehyde. Measurements showed that about 45 c.c. distilled.

The above averages indicate that, with the quantities of permanganate and formaldehyde used in these experiments, the best results were obtained by allowing 30 minutes to complete the reaction. When more than that is taken, the amount of formaldehyde liberated seems to decrease and the amount of carbon dioxide to increase. With less than 30 minutes to complete the reaction, the amount of formaldehyde decreases and the amount of carbon dioxide seems also to decrease. Determinations of both carbon dioxide and formic acid are now being made. Most of this work was done with Mr. Rodney M. West, now chemist in the Minnesota State Food and Dairy Commission.

1. SPUTUM SHAKING AND SEDIMENTING APPARATUS.
2. A RABIES COLLECTING OUTFIT.

BURT R. RICKARDS,  
Director Bacteriological Laboratory, Boston Board of Health.

- I. APPARATUS FOR SHAKING AND SEDIMENTING SPUTUM SPECIMENS.

*A. The shaking of sputum.*—Anyone who has had occasion to make many examinations of sputum for tubercle bacilli appreciates the difficulty in uniformly securing a good even smear, thick enough to present a fair sample of the particular specimen under examination, yet not so thick as to obscure the light or jeopardize the chances of finding the organism if present. If the specimen be chiefly mucous in character the difficulty in obtaining caseous particles imbedded therein is greatly increased, and often it is only by the exercise of the greatest patience and care in manipulation that satisfactory results are obtained. One often has an uncomfortable feeling, in cases where the clinical signs are fairly well marked but where bacteriological results are negative, that the organisms are probably present in the lungs, but are being raised in only very small numbers and have escaped observation.

To overcome the difficulty in smearing and at the same time to increase the efficiency of the examination of sputum, it occurred to the writer to investigate the value of digesting, shaking, and sedimenting the specimens.

It was at once apparent that unless the entire process was carried out in the small one-ounce bottles supplied by the laboratory, manipulations would become tedious and confusion of samples might take place. Moreover to secure a complete breaking up of all particles and a resulting homogeneous mixture a rapid short stroke was necessary. A vertical position for the bottle and for the direction of the stroke had the advantage of being convenient and at the same time gave more distance for the contents of the bottle to travel than a simple horizontal motion. Since all shaking machines on the market gave either a long stroke, worked horizontally, or were unavailable for the purpose in hand, the following apparatus was devised:

On an iron platform 14 x 10 inches is mounted a steel shaft running from the center lengthwise to one end, where a heavy flywheel is hung, the platform being raised from the floor by short iron legs. In the center of the platform a truncated metal cone is bolted the two connecting surfaces having a ground joint.

On the top of the truncated cone a steel cylinder is mounted by means of a threaded joint, the cylinder being capped by a steel disc riveted to the sides. The center of this disc or plate is cut out to receive a piston-rod of about the same length as the cylinder. The piston-rod is attached at its lower end to a connecting rod, which in turn is joined to the shaft. The lower joint runs in oil, while the upper joint is splashed with oil when the machine is running. About midway of the piston rod a plate is attached which fits the cylinder closely and which supports a flat steel spring. As the piston rises, air is compressed between this plate and the cap of the cylinder and escapes through several small valve holes in the sides, this together with the spring reducing the jolt of the upward stroke to a minimum.

A second-steel cylinder, to which is attached a broad platform carrying small metal cups, slides upon the inner cylinder. The top of the outer cylinder is capped and the cap is bolted to the top of the piston rod, an up-and-down motion thus being conveyed to the outer cylinder and the platform. As the outer cylinder rises, an air space is formed between the cap of the outer and that of the lower cylinder. The jolt of the downward stroke is thus absorbed in the same manner as that previously described for the upward stroke. The machine can be either directly connected with a small motor, or belt driven. A speed of 500 strokes per minute for from five to ten minutes has been found satisfactory.

At one side of the platform a short lever is provided to drive the stoppers of the sputum bottles home, the same level being obtained with each one by means of a small stop fitted with a milled thumbscrew. The bottles fit snugly in the metal cups provided on the platform, and are held in place by a heavy metal cap-plate which fits over the top of the outer cylinder and which is fastened down by two small thumbscrews.

By means of this apparatus all masses coagulated by the 5 per cent carbolic acid sent out in the bottles and all caseous particles in the sputum are rapidly broken up and the sputum rendered homogeneous throughout. The time necessary to smear a dozen specimens properly is materially reduced, and the smears are more even and less apt to flake.

A sufficient number of comparative tests has been made to show that the total number of positives is increased somewhat by this method.

Out of 1,000 specimens examined 212 were positive both before and after shaking. Four samples gave positive results before shaking and negative results after, while 10 gave negative results before and positive results after shaking.

In other words there is a loss of about 1.7 per cent in positive

results by shaking, but also a gain of about 4.5 per cent, giving a total gain of about 2.8 per cent.

The only explanation for the decrease after shaking that the writer has to offer is that possibly if only a very few bacilli are present, say, for instance, in a single caseous particle, this might be fished for and obtained before shaking, but the organisms after shaking might be so scattered as to be missed entirely.

Note was made in every case where there was a comparative increase or decrease in the number of bacilli present. In 29 cases there was an increase and in 10 a decrease in the relative quantity of organisms present.

One would naturally not expect the increase in the total number of positive specimens to be large, since a considerable number of specimens are undoubtedly submitted on very slight suspicion, thus giving a large percentage of negatives. On the other hand a diagnosis made earlier than was hitherto possible, even if on only a very few samples, is of considerable value to the persons concerned, in the light of our present knowledge.

If a small amount, from 1 to 2 per cent, of a 10 per cent aqueous solution of caustic soda is added previous to the shaking, digestion of the mucous takes place very quickly, rendering the smearing, etc., still easier. Such digestion is necessary if the sputum samples be sedimented.

*B. The sedimenting of sputum.*—The problem of properly sedimenting the sputum in the original bottles was far more easily solved than that of shaking. Any Babcock machine having a speed of 1,200 revolutions per minute or over does fairly well, provided the sputum bottles used are small enough to fit into the Babcock cups. More uniformly satisfactory results can be obtained with a speed of 2,000 or over. In the machine used by the writer the cups are held in place by trunnions which fit into the slots in a metal head or pronged disc. The regular cups can thus be taken out and special cups of the exact size to receive the sputum bottles inserted.

Lack of time has prevented the presentation at this time of some of the minor details of the method, such as the most advantageous amount of digestive solution to add, the speed, and length of time necessary to obtain the best results. It is the intention of the writer

to determine these points and to secure evidence as to the relative efficiency of this as compared with ordinary methods of examination. Enough has been done to indicate that very satisfactory results may be obtained.

## II. A COLLECTION CASE FOR RABIES SPECIMENS.

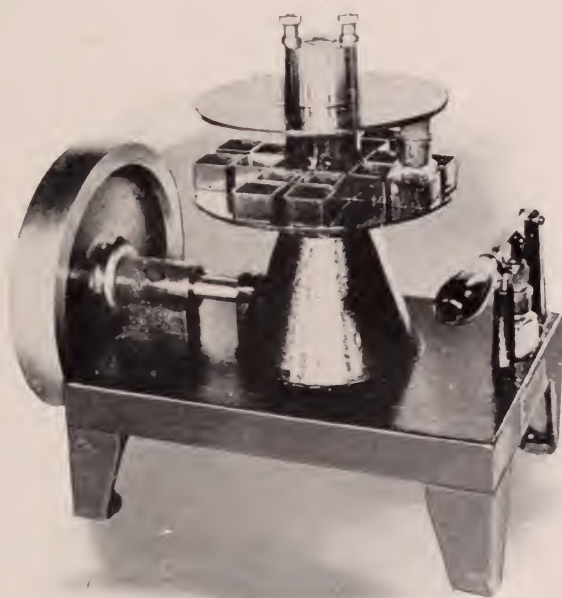
Rabies first developing in the western part of Massachusetts in the fall of 1905, and becoming epidemic in Boston in the spring of 1906, it became necessary to have some convenient way of severing the head of the supposed infected animal at the spot where it was killed and of conveying the same back to the laboratory. The following answers the purpose well:

A tin-lined copper box 18 inches long by 8 inches wide and  $9\frac{1}{2}$  inches deep is divided off at one end into two small compartments. These two compartments are made by running a partition  $3\frac{1}{2}$  inches from the end of the box and a second lengthwise partition  $3\frac{1}{2}$  inches from the front of the box. The smaller space is designed to carry a one-pint bottle of 5 per cent carbolic acid. The second small compartment is a convenient place for rubber gloves, a bottle of chloroform, etc. The remaining large compartment will easily hold the head of a Newfoundland, St. Bernard, or other large dog. The hinged cover of the box is heavily reinforced and fits down upon a wired edge. On the inner part of the cover are metal clamps, holding a saw and dissecting knife. When closed the cover is held in place by two sliding clasps and a hasp and padlock. A tubular brass handle is firmly fastened to the cover. The complete case is of suitable size and weight for easy transportation. As every part of the box is of metal it can be sterilized by placing on a piece of sheet iron over a two-burner gas stove, filling the box partly full of water and boiling.

When many cases are to be examined, the convenience of such a box as that just described is evident. An untrained laboratory assistant may be sent out with the box, the head of the suspected animal removed, placed in the box, and brought back to the laboratory in public conveyances, if necessary, without the delay of waiting for the express, and without the necessity of disposing at the laboratory of the rest of the carcass.



PLATE I.





## A NEW MOSQUITO CAGE.

MARSHALL LANGTON PRICE.

Baltimore, Maryland, U. S. A.

THE mosquito problem is becoming an important one throughout practically all of the western hemisphere, as well as in large areas of Europe, Asia, Africa, and Australia. I do not refer alone to that portion of North America lying between the thirtieth parallels of north and south latitudes. In these regions the normal habitat of the *Stegomyia fasciata*, endemic and epidemic foci of yellow fever make the problem not only important, but urgent; in fact, a large part of the energies of sanitary administrations in Cuba, Mexico, and the Gulf states of the United States, must be directed toward the destruction of mosquitoes and the prevention of their breeding. There are evidences, however, that this problem, now mainly the problem of the hygienist of the *Stegomyia* area, must be met also by the hygienists of all areas where diptera normally or occasionally occur. Mosquitoes are now entering public hygiene in the United States, not alone in the relatively insignificant "malaria area," in the rôle of "unsanitary nuisances."

Although all varieties of the common *Culex* and *Anopheles* have been proven to convey disease, the people of Northern American latitudes are more directly interested in the problem from the standpoint of public comfort. In certain areas, notably on the Atlantic coast of New Jersey and New York, the mosquito is an important economic problem, and its extermination in these states has repaid many fold the expenditure of public money through increased value of the land. The extermination of mosquitoes over any large area, or upon any extensive scale, requires careful study and a definite plan of campaign. An absolutely essential preliminary, is a geographical survey of the area involved, to determine the varieties of mosquitoes to be dealt with, and their distribution. In fact the sanitary laboratory in the near future will have to make studies of variety and distribution of mosquitoes with the same care and attention as is now given to pathogenic organisms. For this purpose it is necessary to collect,

raise, and observe mosquitoes in the laboratory. The study of mosquitoes includes the collection of eggs or larvae, hatching in water, and the preservation and observation of the imagines in suitable cages. The cage here described was devised with the object of facilitating the raising of mosquitoes and their observation during hatching, and in the imago stage. As a preliminary to experiment, observation, and diagnosis of variety, eggs or larvae must be collected, and transferred to hatching-jars. The larvae may be collected by means of a fine net passed through any pond which may breed mosquitoes, well below the surface of the water. Eggs may be easily recognized by a careful observer, and transferred by means of a spoon. The eggs or larvae are transferred to a hatching-jar filled with rainwater until they have fully developed.

The usual method of developing imagines is to hatch the eggs or larvae in jars protected with netting, and to transfer each adult insect separately by means of a short test-tube to cages of wire netting. In order to avoid the laborious handling of each single insect, to avoid loss during transfer, and to facilitate observation and experiment, the larvae jars have been combined with the imago cage in the manner illustrated in the drawing.

*Description of the cage.*—The cage is of wood, divided into three compartments by partitions. The base is made of a single piece of wood and supports the two vertical wooden partitions and two end pieces. A floor separates the larvae jars from the imago compartments. The top of each imago compartment is closed by a piece of plate glass, held lightly by screws. The sides of the imago portion of the cage are closed by wire and cloth netting (a double layer) held in place by brass upholstery tacks.

The two vertical partitions divide the space between the base and floor into three compartments, each made to fit accurately one of the larvae jars, so that the latter can be slid easily in and out of their compartments without allowing any interval through which the insects can escape when the jar is in place. Each larvae jar communicates with its imago compartment by an opening slightly smaller than the mouth of the jar. Through this opening the imagines pass upward into the netting cage as soon as they undergo their metamorphosis.

The partitions and ends of the cage are supported above by two longitudinal wooden strips each  $1\frac{1}{2}$ " wide, mortised into the partitions and ends. All of the wood used in the construction of the cage is  $\frac{1}{2}$ " pine.

As it is important for the larvae jars to fit accurately, the wood should be well seasoned and given a thorough rubbing with an oil filler.

The cage, including the netting, should be painted inside and out with a white enamel paint, which will add materially in observing the mosquitoes.

The plates inclosing the tops of each compartment are a good quality of plate glass,  $\frac{1}{8}$ " thick, and bored for from three to six 0.2" screws.



The two end plates are  $9\frac{11}{16}$ " by  $8\frac{3}{16}$ ". The plate closing the central compartment is  $9\frac{1}{8}$ " by  $8\frac{3}{16}$ ", and the feeding plate of the same dimensions.

The full size of the cage is, height 18 inches, width 8 inches, length 28 inches. The larvae jars are  $2\frac{1}{2}$ " high, and the diameter of their mouths  $4\frac{1}{2}$ ". Their compartments correspond in height, but are somewhat larger in the other dimensions. A space is thus allowed on the floor of the imago compartment upon which the mosquitoes can rest, and upon which their food (dates, bananas, sugar, and water, etc.) is placed in small butter plates.

*Feeding-plate.*—If it is desired to propagate mosquitoes through several generations, or to persuade fecund females to lay, or to carry

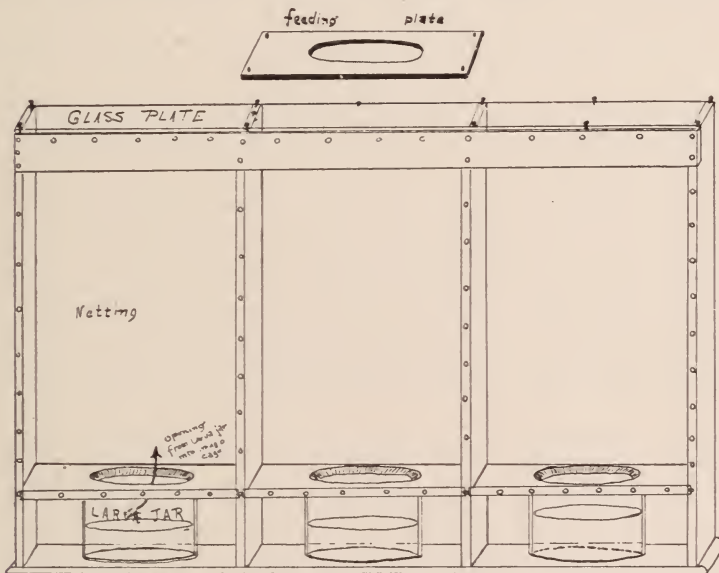


FIG. 1.

out any line of experiments in diseases for which the mosquito is the intermediate host, provision must be made to furnish the impregnated female insects with blood. This is absolutely essential in the case of the *Stegomyia*, as the female will not lay fertile eggs (if at all) without the previous ingestion of blood, preferably from a human source.

The usual method of furnishing blood to insects in cages, is to provide a special sleeve of netting, protected with a draw-string through which the arm can be introduced into the cage. There are two serious objections to this method. One is that the string produces folds in the netting into which mosquitoes will frequently squeeze themselves

and die, and the other is the impossibility of observing the insects during the act of sucking blood. In the present cage the netting sleeve is replaced by a feeding-plate. This plate is of glass with a central elliptical opening made to fit the forearm of the experimenter at its largest part. A paper or cardboard pattern should be made for the feeding-plate and for the three plates closing the top of the compartments, to show their size, the position of the screw holes, and the position of the central opening in the feeding-plate.

To make the pattern for the feeding-plate, a piece of paper or strathmore board should be cut to the exact size of the plate and the center marked by means of two diagonals. The major and minor axes of the forearm should be determined by means of a pair of compasses or calipers. Draw the major and minor axes intersecting the center at right angles to the sides and with two pins attached to a piece of string, the length of the major axis, draw the ellipse. The pins should be placed equidistant from the center, at such a distance that the curve will just intersect the ends of the axes.

The feeding-plate now in use in the cage was bored and cut to fit the pattern of the forearm by a Baltimore firm dealing in plate glass, but it could probably be made more economically by etching with hydrofluoric acid. In this case it could easily be made an exact fit of the forearm of the experimenter. In this case the ellipse could be described as already stated. After taking the axes of the forearm and describing the ellipse, the forearm may be measured with a piece of lead tape, drawn over the ellipse, and the pattern cut out on a piece of paraffined paper. By pouring hydrofluoric acid upon the opening in the paper and using if necessary several pieces of paper, a hole can be etched which will fit accurately the arm from which the pattern was made.

*Method of using the feeding-plate.*—The feeding-plate may be used, when it is necessary to supply the impregnated females with blood, in the following manner. This involves the substitution of the feeding-plate for the plain glass plate closing the top of the compartment. To do this the screws are removed from the top plate and the feeding-plate placed against its edge and gradually pressed against the top plate until it has replaced the latter, sliding off at the same time the top plate. The opening in the feeding-plate is closed during this manipulation by one of the covers ground to fit the top of the larvae jars. When the feeding-plate is *in situ* and lightly fastened with screws, the cover is removed, and the forearm inserted. If the opening does not fit accurately a piece of gauze is wrapped around the forearm below the elbow. This glass plate allows an uninterrupted view of the females during blood-sucking.

The cost of the cage with the glass plates is about \$15. The largest part of the cost is in the glass plates, but the increased facility of observation more than compen-

satés for their cost. These plates cost in Baltimore \$1.50 each for the plain plate-glass top plates, and \$5 for the feeding-plate. The cost of the latter is mainly in the feeding-opening, as the boring and cutting of these plates is a difficult and delicate operation.

The larvae jars are the cylindrical jars with ground covers used for instruments and dressings, and cost 50 cents each. Their size is  $2\frac{1}{2}$ " by  $4\frac{1}{2}$ ". The wooden foundation may be made by any intelligent carpenter. This portion of the cage cost \$2.50. The netting can be applied in half an hour by anyone. An inner layer of fine mesh-cloth netting and an outer layer of wire netting were used in this cage.

The glass feeding-plate will be found especially valuable in removing adult insects from the cage, as it permits binoptric vision, which is very difficult if netting or a gauze sleeve is used. There being no netting in the top of the cage, a full and unobstructed view of the imagines is always available. The combination of the larvae jars with the imago cage relieves the experimenter from any close care and observation other than the maintenance of a proper temperature and the supplying of the imagines with food and blood when necessary.

The larvae jars may be removed from the cage at all times, excepting when the pupae are undergoing metamorphosis. By placing the jars upon a white or neutral surface, nearly all the details of structure necessary for diagnosis may be observed without magnification.

The total weight of the cage, including water in the jars, is less than ten pounds, and it can be easily moved about for observation and demonstration.

With the cage made in three compartments, as in the experiments for which it was devised, two separate methods of treatment and one control were provided for, but any number of compartments can be constructed, or a unit plan may be employed.

# THE ACTION OF SUNLIGHT UPON BACTERIA WITH SPECIAL REFERENCE TO *B. TUBERCULOSIS*.

JOHN WEINZIRL.

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## INTRODUCTION.

THE action of sunlight upon living bodies has always been a problem full of interest to the biologist, and even to the chemist and the physician. The well-known effect upon green plants in which carbon dioxide from the air and water from the soil are gradually changed, through the influence of sunlight, into starch, cellulose, and sugar, naturally led many to believe that sunlight exerted an important influence upon other life as well. With respect to the animal species we know comparatively little, only the lower members, as the protozoa, having been extensively studied; on the other hand, the lower plant forms, especially the colorless plants, early received systematic investigation. The bacteria received the most attention, undoubtedly because of their relation to disease and to national and household economy. The new methods of work which bacteriology originated promoted this sort of investigation; as a result, we possess today an extended literature upon the question of the action of light upon the bacteria. This action is described as bactericidal, the non-spore-

forming species being killed in about one and one-half hours in summer, and two and one-half hours in winter.

One phase of this subject appears, however, to have received little or practically no attention; this is the effect that light exerts upon the bacillus of tuberculosis. When it is recalled that tuberculosis carries off one-seventh of the human race, not to mention the accompanying suffering and financial sacrifices that its long-drawn-out character entails, it appears that this organism should have received early and abundant attention. The cause of this neglect is not difficult to understand. In the first place, the tubercle bacillus is a slow grower when compared with most other bacteria. A day or two will give satisfactory growths with many, while the tubercle bacillus requires two to four weeks. In the second place, tubercle bacilli will not grow on the ordinary media, but require proteids such as coagulated blood serum or egg albumen. The temperature, too, must be held rather closely to 37° C., and the moisture must be carefully conserved. Taken together, these facts have evidently deterred workers in this field from employing the tubercle bacillus.

The question of the action of sunlight upon *B. tuberculosis* was taken up by the writer in the summer of 1905. It was soon found, however, that the conventional methods for testing the action of sunlight upon bacteria were not feasible in working with *B. tuberculosis*; and, indeed, this is another reason why so little work has been done upon this important organism. In attempting to determine a suitable method, it was found that the slow-growing character of the culture made it unsuitable for the rapid testing of new methods; other bacteria were employed for this purpose, *B. coli* answering the purpose admirably because of its vigorous growth, its heavy cloudiness in bouillon, its resistance to desiccation, and, more especially, the readiness with which it could be identified when occasion demanded. Consequently this organism has been used in a large part of the work, and the report made to include these tests. As will be seen presently, this appeared most desirable, for the application of new methods required a comparative study, and this study at once showed certain defects in all the data that had heretofore been gathered. Before proceeding farther, we may with advantage review these data briefly; not that they are few, for they fill many pages, and a simple bibliog-



raphy would include perhaps a hundred titles; nor, indeed, because they are unimportant, for they have served as necessary stepping-stones in our progress.

For bibliographies, the reader is referred to the publications of Marshall Ward, Dieudonné, and Raum. Here only the epoch-making researches, and such as bear directly upon the present line of work, need be considered. These will be presented in some detail in order that direct comparisons may be made with the work presently to be recorded.

#### HISTORICAL REVIEW.

A. *Action of light on bacteria in general.*—The fact that light exerts a detrimental influence upon the colorless plants must have been recognized at an early date; it was most natural, therefore, when bacteriological methods were sufficiently developed, that considerable attention should have been paid to this group. Within a decade after Koch's discovery of the solid media, many of the important species were tested as to the action of sunlight upon them, and the results recorded. The past decade has added little that is new to this field, save the testing of X- and Radium rays.

The earliest work, preceding even the days of solid media, is that of the now classical investigations of Downes and Blunt.<sup>1</sup> They were, indeed, the first workers in this field. They exposed broth cultures containing a mixture of organisms, and observed a decided inhibitive action. Days or even months were required to sterilize the cultures.

Another early worker in this field was Arloing.<sup>2</sup> He used cultures of anthrax, and came to the conclusion that if spores were present they were not killed out until 25 to 30 hours had elapsed. In an earlier trial, broth tubes containing spores, which had been exposed for two hours in July, remained sterile. If allowed to germinate, 25 to 30 hours were required to kill.

Using one of his *Tyrothrix* forms which produced spores, Duclaux<sup>3</sup> found that in bouillon tubes:

after 5 days' exposure	1 out of 3	remained sterile
" 1 month's "	2 " "	3 " "
" 2 months' "	3 " "	3 " "

When micrococci were employed<sup>4</sup> the time required to kill them was decidedly less, not more than 40 days in the spring time, or 20 days in summer. If exposed in a dry state, eight days in spring and two to three days in summer sufficed to kill.

Marshall Ward<sup>5</sup> also worked with anthrax, but substituted agar plates for bouillon tubes. Gelatin was tried, but this medium melted in the sun, and so gave uncertain results. His agar plates were covered by a black stencil. The results were not very definite, and varied with the brightness of the days. In March, exposures of one-half

<sup>1</sup> *Proc. Roy. Soc. Lond.*, 1877, 26, p. 488; and 1878, 27, p. 199.

<sup>2</sup> *Comp. rend. de la Acad. Sci. Paris*, 1885, 100, p. 378, and 101, pp. 511, 535.

<sup>3</sup> *Ibid.*, 100, p. 119.

<sup>4</sup> *Ibid.*, 101, p. 395.

<sup>5</sup> *Proc. Roy. Soc. Lond.*, 1890-94.

to one hour inhibited the growth; and one to one and one-half hours killed. He tested the action of the various colored rays, with the general result that the more refrangible (green to violet) rays showed germicidal powers upon both spores and bacilli.

Working about the same time as Ward, we find Buchner,<sup>1</sup> whose classical demonstration of the sun's bactericidal action on a typhoid agar plate is so well known as scarcely to require mention. The plate was covered with black paper through which the letters of the word "typhus" were cut; this plate was exposed to direct sunlight and then developed in the dark. In the letter openings the bacteria were killed, and when the paper was removed, the letters stood out boldly.

In another experiment he made an aqueous suspension of *B. coli* containing 100,000 germs per c.c. After exposing to direct sunlight for one hour, the suspension was found to be sterile.

Dieudonné<sup>2</sup> experimented with *B. prodigiosus*, and *B. fluorescens putidus*, especially because they were supposed to be highly sensitive to light-action. He found that in agar and gelatin plates, one and one-half hours of exposure kill the bacteria in March, July, and August; while two and one-half hours were required in November. Repeating the work with *B. coli*, *B. typhosus*, and *B. anthracis*, substantially the same results were obtained. He also investigated the effect of the different rays by the use of colored solution screens, and by the prism with an arc light. Both confirmed Ward's work. As to the question whether the action was primarily upon the medium and so indirectly upon the bacterium, or upon the organism direct, he believed the latter was mainly the case, although the medium was somewhat changed.

Kruse<sup>3</sup> worked more especially upon secondary questions, such as the effect of the different colored rays, using colored solutions for his screens; also the influence exerted by the presence of gases, such as hydrogen and oxygen. In oxygen the bacteria, when exposed to sunlight, were killed in three hours, while in hydrogen they were still living after six or seven hours. He also tested the action upon the culture medium; while this was affected, the growth being somewhat inhibited, still this was not sufficient to explain the action of sunlight upon bacteria.

Recently some experiments were made at Lawrence, Mass., by Clark and Gage.<sup>4</sup> The typhoid and colon bacilli were employed, watery suspensions of fresh cultures were made, and 1 c.c. of the suspension was placed in a petri dish which was then exposed to direct sunlight. After exposing a definite time, agar was added to the water and the plates incubated. In 10 to 15 minutes' exposure, 95 to 99 per cent of the typhoid germs were killed, and 96 per cent of the colon germs. To destroy completely all life, the time varied from one-half hour up to four hours. These tests were apparently not repeated except in a modified form in one or two instances.

*B. Action of light on B. tuberculosis.*—Coming now directly to the effect upon the tubercle bacillus, we have scanty data to review. The earliest observations were made by Koch whose remarks at the Berlin Tuberculosis Congress, 1890, evidently made from general observations upon this subject, have found their way into so many articles and textbooks as follows:

"The tubercle bacillus is killed quite rapidly by light. A few minutes' to several hours' direct sunlight kills. Diffuse sunlight, though slower, gives the same result. Tubercle cultures set by the window die in five to seven days."

<sup>1</sup> *Centralbl. f. Bakt.*, 1892, 11, p. 781.

<sup>2</sup> *Arch. a. d. kais. Gsundtsamt.*, 1894, 9, pp. 495, 537.

<sup>3</sup> *Ztschr. f. Hyg.*, 1895, 19, p. 313.

<sup>4</sup> *Rep. Mass. State Board of Health*, 1902, p. 245.

As a result of these remarks, a general impression seems to have gone out that the tubercle is exceedingly sensitive to the influence of light, a point to which reference will be made later.

Frequent reference is also made to the work of I. Strauss,<sup>1</sup> who, after quoting Koch's remarks as given above, says:

"I have been equally able to assure myself that some cultures with abundant human and avian bacilli, developed upon the surface of glycerin bouillon in glass flasks, were killed after having been exposed upon a balcony for two hours to the rays of the summer sun. Besides these flasks I exposed to the sun some cultures previously dried in thin layers on some glass plates; already, at the end of half an hour, they had lost their vegetative function and their virulence."

The work of Migneco<sup>2</sup> also requires notice, although it is of a limited character. He smeared sputum, known to contain numerous tubercle bacilli, upon various kinds of cloth, hung the cloths so as to be exposed to sun and wind, and after various intervals of time, strips (2×5 cm.) were cut off and inoculated subcutaneously or intraperitoneally into guinea-pigs. He concludes that sunlight has a detrimental effect upon tubercle, as well as upon other bacteria, and that upon linen or woollen cloth it can withstand sunlight not more than 24 to 30 hours, provided that the layer of sputum is not too thick; also, the virulence of the bacilli is greatly weakened after 10 to 15 hours.

A number of men have carried out practical tests similar to the above, but the work scarcely requires mention here.

C. *Observations on recorded data.*—Enough has been said to show the strictly limited character of the experiments, and the totally inadequate data for any accurate conclusion to be drawn from them, so far as tubercle is concerned. It will also be observed that in all the experiments (save Migneco's), the various kinds of bacteria were exposed under glass, i. e., in glass vessels. It is a fact well known to physicists that glass reflects and absorbs a large proportion of the sun's rays, depending upon conditions. If the light strikes the surface quite obliquely, then by far the larger portion is reflected; if it strikes vertically, then a considerable amount is absorbed by the glass itself. Again, the bacteria tested have always been planted in or upon some medium (one experiment of Strauss's excepted). These media are always slightly colored or clouded, and consequently absorb even a larger proportion of the light than the glass. In some experiments, the heaping-up or clumping of the organisms, as in naturally grown cultures, or in sputum smears as used by Migneco, the data are totally invalidated save from a most limited point of view. Taken together, these various factors have discounted the effectiveness of sunlight as a germicidal agent, varying from a fourth to perhaps nine-tenths, or even more in some cases. These shortcomings in methods of work the writer has succeeded in completely overcoming, but only after a number of months of laborious work.

Hence the desirability of more adequate and accurate data concerning the action of sunlight upon the bacillus of tuberculosis goes unquestioned. It would seem, too, that a general reopening of the question of the action upon bacteria in general is not only justified, but is most highly desirable, especially in view of the rapid strides that sanitary science is making at the present time.

<sup>1</sup> *La tuberculose et son bacille*, 1895, p. 220.

<sup>2</sup> *Arch. J. Hyg.*, 1895, 25, p. 361.

## A STUDY OF METHODS

Even at the beginning of the work, or rather as a result of some work done along this line in New Mexico, it was recognized that new methods were necessary, if the data are to have the highest value. This study of methods took two directions: first, an attempt to find a method that could be used with *B. tuberculosis*, since the methods usually employed fail utterly when applied to this organism; secondly, an attempt to minimize or eliminate completely the absorption factor of the interposed glass and media. These two lines of work were developed simultaneously, and it will not be convenient to separate them in the discussion, for, as will be seen, each helped to solve the other.

A. *Exposure of organism on media*.—As frequently happens, this work was begun at the most difficult point, viz., the methods for tubercle. Dorset's egg medium<sup>1</sup> furnished the most ready and satisfactory method of growing pure cultures of tubercle, and consequently this was selected for the work at hand. The plate method of exposure as commonly practiced was not suitable, because of the rapid desiccation incident to incubation. The most natural suggestion was to employ sloped test-tube cultures, similar to agar slopes, and expose these. Dorset's glycerine-phosphate agar was tried in the same way.

On August 19, 1905, a large number of cultures, made as suggested above, were exposed to direct sunlight for one-half, one, two, three, and five hours respectively. Two strains of tubercle bacilli were used:

*Culture No. 101*,<sup>2</sup> human type, was isolated at the local laboratory from human sputum, and was of fully average virulence.

*Culture No. 110*, from bovine source, was isolated by Dr. E. L. Baldwin of Saranac Lake. The virulence of this bacillus, when tested by the writer, was certainly not greater than that of No. 101, and was considerably below what is usually considered as characteristic for the bovine variety.

In this connection the descriptions of the additional varieties subsequently used may also be given.

*Culture No. 102*, was obtained from the Bureau of Animal Industry, U. S. Department of Agriculture, where it was used in making tuberculin. It possesses very low pathogenic powers, and grows rapidly and abundantly on egg medium.

*Culture No. 113*, avian, was isolated by the writer from a tuberculous hen in July, 1905. It grows more sparingly than the two human cultures, the colonies being more restricted and elevated.

<sup>1</sup> *Bull. Bur. Anim. Indus.*, 1904, 52, Pt. I.

<sup>2</sup> The numbers are those in use at the local laboratory, and have no special significance.



These bacilli, taken from young cultures, were inoculated upon the sloped media in test-tubes, and exposed to the direct rays of the sun on a wire support raised about eighteen inches from the floor. In this and all subsequent experiments the attempt was made to keep the sun's rays vertical to the surface of the medium; this was impracticable except when employed as an approximation toward the ideal. In addition to the egg and agar cultures, a watery suspension was exposed in a test-tube similarly to the others, and cultures made at intervals. Another variation consisted in rubbing the bacilli on small strips of sterile cloth, exposing these in a petri dish, and then inoculating the cloth and bacilli into an egg tube.

Substantially the same series of experiments was repeated on September 8, substituting paper for the cloth, however, and inoculating this upon egg. The results of these experiments are embodied in Table 1 below:

TABLE 1.  
EFFECT OF SUNLIGHT UPON CULTURES OF *B. tuberculosis*.

EXP. No.	DATE	No. OF CULTURE	MEDIUM USED	RESULT OF EXPOSURE (HOURS)	
				Growth	No Growth
1.....	Aug. 19, '06	101	Egg	$\frac{1}{2}$ , 1, 2, 3	5
2.....	" 19, '06	101	Agar	$\frac{1}{2}$ , 1, 2,	3, 5
3.....	" 19, '06	101	Aqueous suspension	$\frac{1}{2}$	1, 2, 3, 5
4.....	" 19, '06	101	Cloth	$\frac{1}{2}$ , 1, 2, 3	5
5.....	Sept. 8, '06	101	Egg	$\frac{1}{2}$ , 1, 1 $\frac{1}{2}$ , 2, 3	.....
6.....	" 8, '06	101	Aqueous suspension	$\frac{1}{2}$	1, 1 $\frac{1}{2}$ , 2, 2 $\frac{1}{2}$ , 3
7.....	" 18, '06	101	Paper	$\frac{1}{2}$	1, 1 $\frac{1}{2}$ , 2, 2 $\frac{1}{2}$ , 3
8.....	Aug. 19, '06	110	Egg	$\frac{1}{2}$ , 1, 2, 3, 5	.....
9.....	Sept. 8, '06	110	"	$\frac{1}{2}$ , 1, 1 $\frac{1}{2}$ , 2, 3	.....
10.....	" 8, '06	110	Paper + Moisture	$\frac{1}{2}$	1, 1 $\frac{1}{2}$ , 2, 3

The data in this table showed a number of interesting points:

a) The method of exposing test-tube cultures is plainly unsuited to this purpose. This is readily understood when it is recalled that many of the bacilli are worked into the egg during the process of inoculation, and are consequently materially protected. The cylindrical surface of the tube tends to concentrate the light in a line and thus produce unequal intensities upon different portions of the surface of the medium. Consequently, this method was immediately abandoned.

b) The method of exposing watery suspensions appeared quite promising, but it was open to the objection of unequal distribution



of light, which is not wholly remedied by placing the suspensions in potato or petri dishes. In addition, there was a marked tendency for the culture to sediment.

c) The method of spreading the culture on cloth and subsequently inoculating upon egg was also unsuitable, for the reason that the culture worked into the interstices of the cloth and so received protection. Data thus obtained are quite worthless, since we have no means of knowing how much of the effect to ascribe to sunlight and how much to the factor of protection.

d) The method of spreading the pure culture upon sterile paper slips and then exposing them in petri dishes gave the same results as the watery suspensions, and at the same time eliminated the objectionable features of that method, i. e., the unequal distribution of light. Accordingly, this method was temporarily adopted in the work, and considerable data collected, before any advance was made.

B. *Exposure of organism on strips of paper under glass.*—The strips of paper used were about  $1 \times 5$  cm. area; they consisted of ordinary, sized paper and were sterilized by heat before using. Such strips of paper are difficult to inoculate when placed in a glass vessel; but this difficulty can be overcome by placing a piece of cloth in the bottom of the dish and laying the paper upon it; the cloth holds the paper in place both during the inoculation and the exposure. Watery suspensions spread very readily upon such sized paper, serving better than many materials subsequently tried.

In applying this method, several important questions arose, which require discussion:

a) *How does the quantity of culture inoculated upon the paper influence the result?* An early instance of the possible variation in result due to this factor was observed. Among a series of cultures, exposed September 8, were two sets on paper slips: one set showed that tubercle was killed between one and one-half and two hours; the other set between one-half and one hour. At that time the method of inoculating the paper slips was to rub upon them some of the culture by means of a stiff platinum wire. Plainly, the amount of culture transferred to the paper varied within wide limits, even when special care was exercised. Portions from different parts of the culture tube would spread very differently, and necessarily required

different periods of exposure to kill, as shown by the instance cited above. Other instances of such variation were observed, but the details need not be given, for the point is perfectly plain. Suffice it to say that steps were immediately taken to remedy this defect. These steps were as follows:

(1) Instead of taking the culture direct, a suspension in plain water was first made, then a small loopful of this suspension was spread upon the sterile paper slips. With most species of bacteria, this method works quite satisfactorily, but this is not always the case with tubercle. Cultures of tubercle vary so widely in their physical condition, depending upon their age and the medium employed, that all grades ranging from a soft, friable to a hard, resistant mass are obtained. These do not work up equally well into a homogeneous suspension, but are apt to produce flocculi which may readily be carried over to the paper. It is obvious that the germs within or under such a lump are protected to a considerable degree from the influence of sunlight. The effort to overcome this difficulty led to the second step:

(2) The filtration of the suspension through glass wool. Necessarily this must be done under aseptic conditions to prevent contamination. The suspension so obtained is much more uniform in character, all the gross lumps and fatty flocculi being removed. It does not completely remove the microscopic lumps consisting of 10 to 50 bacilli, and to break these up still further it is advisable to employ a shaking machine for some time. However, quite uniform results can be obtained without the additional shaking.

b) *How is the inoculation of the media with the cultures on paper to be accomplished?* This was done in a simple and surprisingly satisfactory manner by placing the paper by means of a sterile forceps on the egg slope. At first an attempt was made to dislodge the bacilli from the paper, but it was soon found that the culture grew admirably upon the paper if it was carefully pressed upon the medium and sufficient fluid was present to moisten the paper. The nutrient medium filters through the paper and nourishes the bacteria, which grow in a wholly satisfactory manner, as shown in Pl. 2. The method is applicable to agar slopes also, if care is taken to have some expressed water present; but obviously, old culture media work unsatisfac-

torily. When experimenting with bacteria which grow well in broth or other liquid media, the method becomes the acme of simplicity; the slip of paper is dropped into the tube of bouillon or milk, placed in the incubator, and the characteristic growth phenomena noted.

c) *Is it possible to diminish the absorption of light by substituting a more suitable medium for the glass?* Pure quartz plates would absorb less light than common glass, but such plates are expensive and practically out of the question. Gelatin films and mica plates next suggested themselves, and these were accordingly tried. On October 28, cultures of Tubercle No. 101 were exposed on paper under these covers, and also under glass. The results showed that:

1. Under glass, tubercle was killed between 0 and  $\frac{1}{2}$  hrs.
2. " mica, " " " " "  $1\frac{1}{2}$  " 2 "
3. " celluloid, " " " " "  $\frac{1}{2}$  " 1 "

Apparently there was no advantage to be gained by the use of either mica or celluloid. Before the test was repeated, the problem was solved by rendering it non-existent as will be shown later. The whole question might well have been ignored, but for the fact that it illustrates nicely one of the steps in the progress of the work.

d) *What part of the bactericidal effect is due to desiccation?* Considerable indirect evidence, gained from the earlier experiments, indicated that it was a minor, if not a negligible, factor.

A direct experiment, made December 30, gave the following results:

TABLE 2.

	ORGANISM	MOISTURE ADDED	RESULT OF EXPOSURE (MIN.)	
			Growth	No Growth
1.....	<i>B. coli</i>	—	..	3, 6, 10, 15, 20, 30
	"	+	3	6, 10, 15, 20, 30
2.....	<i>B. typhosus</i>	—	..	3, 6, 10, 15, 20, 30
	"	+	..	3, 6, 10, 15, 20, 30
3.....	<i>M. tetragenus</i>	—	..	3, 6, 10, 15, 20, 30
	"	+	..	3, 6, 10, 15, 20, 30
4.....	<i>B. diphtheriae</i>	—	..	3, 6, 10, 15, 20, 30
	"	+	..	3, 6, 10, 15, 20, 30

It should be noted that the above exposures were made on an unusually clear day, which accounts partially for the short time required to kill the cultures. While, in one instance, the moisture seems to have prolonged the life of the organism, yet, taken as a whole,

the total time involved was so short as to make it quite doubtful whether this was a determining factor.

In another experiment, April 7, an attempt was made to exaggerate conditions by surrounding one set with conc. sulphuric acid, thus rendering the air quite dry, while the other set had sufficient water added to cause a film of moisture to gather under the cover. Under these conditions, both sets were killed within five minutes, the shortest time of exposure.

It appears highly probable, therefore, that the presence of moisture is neither instrumental in killing, nor in greatly prolonging the life of the cultures. Indeed, as we shall see, the method finally adopted totally removes this question from the main problem.

e) *Does the character of the paper used influence the effect of sunlight in killing the culture?* It was stated at the beginning that the paper used was a common, sized writing-paper. It is conceivable that in the process of manufacturing, especially in bleaching, some antiseptic properties were communicated to the paper. To test this possibility, *B. coli* was exposed to sunlight on eight different substances (March 15) with the following results:

TABLE 3.

TRIAL	MATERIAL EMPLOYED	RESULT OF EXPOSURE (MIN.)	
		Growth	No Growth
1.....	Ordinary, sized paper	2, 7	12, 20
2.....	Filter paper	2, 7, 12, 20	.....
3.....	Black porous paper	2, 7, 12, 20	.....
4.....	Parchment paper	2, 7	12, 20
5.....	Aluminum foil	2, 7, 12, 20	.....
6.....	Mica	2, 7, 12	20
7.....	Glass	2, 7, 12	20
8.....	Wood	2, 7, 12, 20	.....

These results appear somewhat surprising at first; but, upon analysis, the reasons for the differences observed are quite plain. The filter paper, the porous paper, and the wood (a soft pine) are sufficiently porous to permit the individual bacilli to enter with the water and thus become sheltered; the aluminum foil, on the other hand, did not permit the making of a uniform spread, the culture drying in patches, which caused a heaping-up effect with consequent protection.



Similar experiments with the same organism gave the following:

TABLE 4.

TRIAL	DATE	MATERIAL EMPLOYED	RESULTS OF EXPOSURE (MIN.)	
			Growth	No Growth
1.....	March 16	Paper previously used	2	5, 10, 15, 20
2.....	" 16	Parchment	2, 5, 10, 15, 20	.....
3.....	" 16	Mica	2, 5, 10	15, 20
4.....	" 16	Glass	2, 5	10, 15, 20
5.....	" 17	Paper previously used	3, 6	10, 15, 20
6.....	" 17	Parchment	3, 6, 10, 15	20
7.....	" 17	Mica	3, 6, 20	10, 15
8.....	" 17	Glass	3, 6, 20	10, 15
9.....	" 17	Filter paper	3, 6, 10, 15, 20, 30	.....

The filter-paper test requires no further comment. Glass shows the same phenomenon as aluminum when not absolutely free from grease; this tendency is shown by the erratic results that it gives. The parchment invariably became roughened and warped in the sterilizing process, which renders it unsuitable for the purpose in hand. There is left to consider only the mica. This was a genuine disappointment to the writer, for the culture spread very evenly upon it. Apparently this should be an ideal substance to use and would serve as a fair check upon the paper method. Upon still other repetitions this continued to give erratic results, and the explanation undoubtedly is to be found in the fact that the surface of a small piece of mica is very imperfect, many cracks extending inward from the cut edges; in these cracks the bacteria evidently found shelter.

Thus the tests fell short of their object, and merely proved the inferiority of these substances for this purpose. Sized paper gives a beautiful spread, as compared with these; there is little tendency to creep into pores, for these are filled by the sizing, and the results are as uniform as could well be expected.

The question with which we started is answered in a measure, at least, by the fact that innumerable control experiments always gave growth, even when the culture remained exposed for several days. Again, *B. tuberculosis* grew admirably upon these papers (see Pl. 2), which certainly contra-indicates the presence of antiseptic principles in material quantities. Finally, as will be seen, cultures exposed without the presence of paper are killed in even shorter periods of time.

*C. Direct exposure of organisms.*—Thus far, the object has been to minimize the absorption factor, and in this way approach more



closely to the true disinfecting action of sunlight. A very material advantage has been gained, *the exclusion of the nutrient medium and the accompanying absorption*. Incidentally this affords another advantage also, viz., the elimination of possible by-products which may be formed in the medium, such as hydrogen peroxide, ozone, organic peroxides, etc., which might aid in killing the cultures and thus complicating conditions, and rendering the action of the sunlight still more problematical. Indeed, it was not certain that any of the disinfecting action could positively be ascribed to the direct action of the sun's rays. To have eliminated these side reactions and at the same time have a method that is applicable in general, for *B. tuberculosis* included, seemed for a time to present the limit of perfection attainable. Nevertheless, the problem of the elimination of all absorption to obtain the true effect of sunlight was not forgotten.

This problem was attacked at the most difficult point. It was known that the tubercle bacillus could be exposed directly, and by use of a suspension which could be inoculated into animals the contaminating factor would be practically negligible. Accordingly, this experiment was tried. A suspension (heavier than usual), of tubercle No. 101, was allowed to evaporate in the bottom of a petri dish, and exposed, uncovered, to the direct rays of the sun. Four or five drops of the suspension were used to insure sufficient material for the inoculation. At the same time, two of the watery suspensions were also exposed in petri dishes, one being covered and the other not, the latter to serve for comparison. These cultures were exposed for one-half, one, one and one-half, and two hours, when they, together with controls kept in the dark, were injected respectively into guinea-pigs, the inoculations being intraperitoneally in all cases.

This experiment was unfortunate in two respects; firstly, the day chosen gradually developed into a hazy one, so that the sunlight was much diminished in power; secondly, a number of the test animals died from an epidemic of disease that affected the stock of guinea-pigs on hand. The guinea-pigs inoculated with the cultures exposed for the longest time, i. e., two hours, all developed tuberculosis and died. The living tubercle bacillus was recovered in pure culture from several animals of this series. The fact that the cultures were alive after two hours' exposure is explained by the diminished sunlight, on

one hand, and by the thickness of the film and the tendency of the suspensions to sedimentate, on the other.

Several months later it occurred to the writer that organisms could be exposed without cover, and all absorption of light be entirely avoided. This method gave most gratifying results as follows:

A suspension of *B. coli* was made, and a loopful spread in a circle in the bottom of sterile petri dishes. (See Pl. 3.) While exposed to the sun, the dishes were uncovered; when taken in, they were again covered, and a layer of lactose litmus agar, which was melted and cooled to 45° C., was spread over the exposed film. The cultures were placed in the incubator for 24 and 48 hours. The first two sets of plates showed that *B. coli* was killed within two minutes. On March 23, the same method showed *B. coli* to be killed in between two and four minutes. March 31, *B. diphtheriae* was killed in between two and five minutes.

The advantages in selecting *B. coli* in the above and subsequent experiments are these: (a) It is not apt to be contaminated with fresh colon bacilli from the air; (b) it grows well as 37°-39° C., while the common air forms that might contaminate the plate do not, as a rule, develop at this temperature; (c) it grows well on lactose litmus agar, while most bacteria do not; (d) it gives red or acid colonies, on this medium, while this would rarely be the case with air germs; (e) if any question arose as to whether a given colony was *B. coli* or not, cultures in glucose bouillon fermentation tubes and in milk would decide the matter, in nearly all cases, the proper gas formula being especially helpful in reaching a decision.

That this method, with suitable variations in media, etc., can be applied to all bacteria, goes almost without saying. Glucose agar will be found best adapted to all acid-producing species, in which case the circle or other character used for the film will be most helpful (see Pl. 3); if only occasional colonies appear they should be isolated and subjected to confirmatory tests. Species that require a highly proteid medium, like *B. tuberculosis*, may be planted upon strips of sterile paper, and after exposure inoculated upon egg or blood serum. Necessarily, a sufficient number of cultures must be made so that one or more of each lot for a given period will come through uncontaminated; on a quiet day this is not at all difficult to accomplish.

*D. Comparison of methods.*—In a certain way, the methods employed have been compared with each other; but it will now be advantageous to make a more direct comparison, especially of data secured with this object in mind. For this purpose only three methods have been considered available; viz.: (a) the agar-plate method; (b) the method of paper cultures under glass; and (c) the method of direct exposure of the bacterial films, no glass or other

medium being interposed. The method of exposing suspensions has not been considered because of its numerous disadvantages.

On March 23, an experiment with *B. coli* partly covering these points gave the following:

1. On paper, under glass, *B. coli* was killed in between 11 and 15 min.
2. " glass, " " " " " " " 10 " "
3. " paper, no cover, " " " " " " " 1 " 2 "
4. " glass, " " " " " " " 2 " 4 "

The absorption of light by the glass is considerable, and in this instance prolonged the life of the bacterium considerably beyond that required to kill without any interfering medium.

April 11, another series with *B. coli* gave the following:

1. On glass, no cover, killed within 2 min.
  2. In agar plate, glass cover, 25 per cent were killed in 10 min.
- |     |   |   |   |   |   |    |   |
|-----|---|---|---|---|---|----|---|
| 85  | " | " | " | " | " | 20 | " |
| 95  | " | " | " | " | " | 30 | " |
| 100 | " | " | " | " | " | 45 | " |

These plates are shown in composite in Pl. 3, Fig 2.

A repetition of the above experiment with other bacteria gave the following results:

TABLE 5.

ORGANISM	DATE	TIME REQUIRED TO KILL	
		By Direct Exposure	In Agar Plates
<i>B. cholerae</i> suus .....	Oct. 12, 1906	Between 5 and 10 min.	In 40 min. 10 per cent killed
<i>B. phosphorescens</i> .....	" 25, 1906	" 1 and 2 "	" " " 95 " "
<i>B. prodigiosus</i> .....	" 30, 1906	" 5 and 10 "	" " " 70 " "
<i>B. Friedländer</i> .....	Nov. 7, 1906	" 0 and 1 "	{ 20 " 75 " "
<i>B. typhosus</i> .....	" 9, 1906	" 5 and 10 "	{ 40 " 100 " "
			" 45 " 90 " "

The time required to kill bacteria is reduced to at least  $\frac{1}{15}$ th, and possibly to  $\frac{1}{20}$ th, of the time required to kill by the old or plate-culture method. These data could be largely extended, but they are sufficient for the present purpose, viz., to show the great advantage possessed by the method of direct exposure. They indicate also the unreliable character of the data thus gathered by the earlier observers, and which have, at most, only a relative value, for only a  $\frac{1}{10}$ th or  $\frac{1}{20}$ th of the sun's disinfecting action has been employed in securing them.

E. *Observations on methods.*—Before leaving the matter of methods a number of minor points may appropriately be mentioned here.

a) Apparently most investigators have paid little heed to the meteorological conditions, or have failed to mention the details. In the present investigation, only cloudless sunlight has been employed, unless otherwise stated. The time has been as near midday as practicable, most of the determinations having been made between 11 and 1 o'clock. In this work the accompanying table has been of material service.

TABLE 6.  
COMPARATIVE LIGHT VALUES\* (EXPRESSED IN SECONDS).

Time of Day	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
8:00.....	10.0	6.0	3.0	2.0	1.7	1.7	1.5	1.7	2.0	3.0	4.0	9.0
9:00.....	4.0	4.0	2.0	1.5	1.2	1.2	1.2	1.5	1.5	1.7	3.0	3.0
10:00.....	2.5	2.0	1.5	1.7	1.0	1.0	1.0	1.0	1.0	1.5	2.0	2.0
11:00.....	2.0	1.7	1.2	1.0	0.7	0.6	0.5	0.7	1.0	1.2	1.7	1.5
12:00.....	1.7	1.5	1.0	1.0	0.5	0.3	0.2	0.5	0.5	1.0	1.5	1.7
1:00.....	1.7	2.0	1.0	1.0	0.7	0.5	0.5	0.5	1.0	1.2	1.5	2.0
2:00.....	2.5	2.7	1.7	1.2	1.0	0.7	1.0	1.0	1.2	1.5	2.0	2.5
3:00.....	4.5	3.5	2.0	1.5	1.2	1.2	1.2	1.5	2.0	2.0	2.5	3.5
4:00.....	9.0	5.0	4.0	2.0	1.5	1.5	1.5	1.7	2.0	2.7	5.0	8.0
5:00.....		60.0	20.0	4.0	2.7	2.2	2.2	2.5	3.0	6.0	20.0	80.0
6:00.....				20.0	15.0	5.0	4.0	5.0	10.0	40.0	70.0	
7:00.....					80.0	20.0	15.0	60.0				

\* By Lieutenant S. W. Very, U. S. N. Printed in *Am. Annual of Photography and Photographic Times Almanac* for 1899.

Table 6 gives the relative light values for photographic purposes expressed as time necessary to expose the plate. All the work has been done at times falling between 0.2 and 1 in this table, thus giving the most intense light obtainable. This affords a certain standard for the work; absolute values, if attainable, would scarcely serve a better purpose.

b) During the warmer days the temperature was taken to guard against the possibility of killing the cultures by heat. It is evident that, in the short exposures as finally practiced, temperature is not a determining factor in any case.

c) Young cultures were used for the exposures; these were usually one or two days old, and rarely more than a week old. In the case of tubercle, the cultures varied from one to two months in age. Controls always served as an additional check upon the vitality of the culture.

#### EXPERIMENTAL RESULTS.

The striking difference in the results obtained by the old methods as compared with those here suggested is best illustrated by a direct comparison of results for specific bacteria.



*B. coli.*

Dieudonné<sup>1</sup> found that agar plate cultures of *B. coli* when exposed to midday sun were killed in 1 to 1½ hours. Buchner<sup>2</sup> found that aqueous suspensions were sterile after 1 to 1½ hours' exposure.

In comparison with these, the writer found the following:

TABLE 7.

DATE	METHOD OF EXPOSURE	RESULT OF EXPOSURE (MIN.)	
		Growth	No Growth
March 20, 1906..	On glass, uncovered	.....	2, 4, 6, 8, 10, 15
" 23, 1906..	" " "	½, 1, 2	4, 7, 10
" 23, 1906..	" paper, "	½, 1	2, 4, 7, 10
April 7, 1906....	" glass, "	2	5, 10, 20, 40
" 11, 1906....	" " "	.....	2, 5, 10, 20, 30

The difference between the above results and those obtained by former investigators is most remarkable.

*B. typhosus.*

Kedzior<sup>3</sup> found that if a 5 c.c. bouillon culture of typhoid was exposed to sunlight, it was not killed in 4¼ hours; even a 1 c.c. culture was not killed in 5½ hours.

By direct exposure this organism was killed as follows:

TABLE 8.

DATE	METHOD OF EXPOSURE	RESULTS OF EXPOSURE (MIN.)	
		Growth	No Growth
April 27.....	On glass, uncovered	2, 5	10, 15, 20
May 10.....	" " "	.....	2, 5, 10, 20, 30
" 15.....	" " "	2	5, 10, 20

The results of April 27, are nearer the others than the figures would seem to indicate, for only a couple of colonies remained on the plate exposed for five minutes. When compared with Kedzior's results, it is seen that the time necessary to kill typhoid by direct exposure is reduced to ½ or less. The advantages of the method of direct exposure are evident.

*B. dysenteriae* (Shiga).

Quite similar to *B. coli* and *B. typhosus* are the results for *B. dysenteriae*:

TABLE 9.

DATE	METHOD OF EXPOSURE	RESULTS OF EXPOSURE (MIN.)	
		Growth	No Growth
April 22.....	On glass, uncovered	.....	2, 5, 10, 20, 30
" 9.....	" " "	.....	2, 5, 10, 15, 20
" 10.....	" " "	2	5, 10, 20, 30

<sup>1</sup> Arb. a. d. kais. Gsndhtsamtl., 1894, 9, p. 405.

<sup>2</sup> Centrbl. f. Bakt., 1892, 11, p. 781.

<sup>3</sup> Arch. f. Hyg., 1899, 36, p. 323.



*S. cholerae-asiaticae.*

In an experiment similar to that with typhoid bacilli, Kedzior found that a 5 c.c. bouillon culture gave a good growth after 4 hours, and a 1 c.c. culture was killed in 5½ hours.

Direct exposure gives the following:

TABLE 10.

DATE	METHOD OF EXPOSURE	RESULTS OF EXPOSURE (MIN.)	
		Growth	No Growth
April 11.....	On glass, uncovered	.....	2, 5, 10, 20, 30
27.....	" " "	.....	2, 5, 10, 15, 20
May 19.....	" " glass cover	1, 2	6, 10, 15

*B. diphtheriae.*

Kedzior also exposed cultures of diphtheria and found them to be killed in 1½ to 2½ hours.

By direct exposure, this organism was killed as follows:

TABLE 11.

DATE	RESULTS OF EXPOSURE (MIN.)	
	Growth	No Growth
March 31.....	½, 1, 1½, 2	5
April 27.....	2	5, 15, 20
May 10.....	2	5, 10, 20, 30

*The Pus Cocci.*

No one seems to have experimented with these forms, hence, no comparison is possible. The results obtained by direct exposure are as follows:

TABLE 12.

DATE	ORGANISM	RESULTS OF EXPOSURE (MIN.)	
		Growth	No Growth
April 2.....	<i>M. pyog. aureus</i>	3, 6, 10	15, 25
" 22.....	" "	2, 5	10, 20, 30
May 15.....	" "	2, 5	10, 20
April 7.....	<i>M. tetragenus</i>	2	5, 10, 20, 40
" 11.....	"	2	5, 10, 20, 30
" 22.....	"	2	5, 10, 20, 30
May 10.....	"	.....	2, 5, 10, 20, 30

In the earlier work two tests were also made with *M. pyog. albus*, but at that time the short period of exposure necessary to kill was not appreciated, and so the results are not very helpful. In the first trial, it was killed within 30 minutes, and in the second within 10 minutes.

*B. prodigiosus.*

Dieudonné<sup>1</sup> studied this organism because he thought it would be especially sensitive to light. He found it to be killed in between  $1\frac{1}{2}$  and  $2\frac{1}{2}$  hours in gelatin and agar-plate cultures.

Direct exposure gave the following:

TABLE 13.

DATE	RESULTS OF EXPOSURE (MIN.)	
	Growth	No Growth
April 27.....	.....	2, 5, 10, 15, 20
May 11.....	1, 2	5, 10, 15

It may be worth noting that, on the two-minute plate of May 11, the color production was not diminished, although the number of colonies was greatly reduced.

*B. pyocyaneus.*

Kedzior worked with this organism and found it was killed in agar plates in between  $2\frac{1}{2}$  and  $3\frac{1}{2}$  hours. By the method of direct exposure, the writer found it was destroyed as follows:

TABLE 14.

DATE	METHOD OF EXPOSURE	RESULTS OF EXPOSURE (MIN.)	
		Growth	No Growth
April 7.....	On glass, uncovered	.....	2, 5, 10, 20
" 11.....	" "	2	5, 10, 20, 30
May 9.....	" paper, "	.....	2, 5, 10, 15, 20
June 30.....	" glass, "	$\frac{1}{2}$ , $\frac{1}{2}$ , 1, 2	4, 10

Two other chromogens were tried, with the following results:

TABLE 15.

DATE	ORGANISM	METHOD OF EXPOSURE	RESULTS OF EXPOSURE (MIN.)	
			Growth	No Growth
April 2.....	<i>Sar. aurantica</i>	On glass, direct	3, 6, 10, 15, 25	.....
May 15.....	" "	" " "	2, 5, 10, 20	.....
" 19.....	" "	" " under glass	10, 20, 30, 40, 60	.....
" 28.....	" "	" " direct	60	90, 120
" 15.....	A pink air-micrococcus	" " "	2, 5, 10, 20	.....
" 19.....	" "	" " under glass	10, 20, 30, 40, 60	.....
" 28.....	" "	" " direct	60, 90	120

These two organisms show a higher order of resistance to the influence of sunlight than those heretofore considered. (See Pl. 3. B.) Indeed, they constitute a group by themselves, to which, presumably, many of the bacteria found in the air belong. Whether the resistance they exhibit is due to a failure to break up the groups, or to a sporelike condition (so-called arthrospore) of the bacterium, remains uncertain. But, from the nature of the suspensions used, and from the character of the growth in the plates, the writer is inclined to hold the latter view.

<sup>1</sup> *Arb. a. d. kais. Gsndhtsaml.*, 1894, 9, p. 405.

It is fortunate that the pathogenic organisms show no such powers of resistance, or the problem of coping with them would be materially more difficult.

*B. tuberculosis.*

Coming now to the bacillus of tuberculosis, which is the chief factor in the present investigation, one finds practically no previous tests that are satisfactory, with which to compare the results. Koch's remarks are seemingly based upon casual observation, which, however, indicated that the organism was highly sensitive to light, presumably more so than other bacteria. Strauss came close to the truth in his trial by exposing a film on glass plates, which he found was killed in 30 minutes. Other workers have used sputum with variable results, ranging from 24 to 30 hours, up to days or weeks.

The earlier results when the bacillus was exposed on egg medium have been given; they are relatively high, varying between two and five hours in time necessary to kill; in aqueous suspensions it was killed in  $\frac{1}{2}$  to 1 hour. The later results, when no culture medium was used in the exposure, are as follows:

TABLE 16.

DATE	No. OF ORGANISM	METHOD OF EXPOSURE	RESULTS OF EXPOSURE	
			Growth	No Growth
September 8, 1905....	101	On paper, under glass	$\frac{1}{2}$ , 1, 1 $\frac{1}{2}$	2, 3, 4, 5, 6 (hrs.)
" 8, 1905....	110	+moisture, on paper, under glass	$\frac{1}{2}$	1, 1 $\frac{1}{2}$ , 2, 2 $\frac{1}{2}$ , 3 (hrs.)
" 8, 1905....	101	" " " "	$\frac{1}{2}$	1, 1 $\frac{1}{2}$ , 2, 2 $\frac{1}{2}$ , 3 (hrs.)
October 28, 1905....	101	On paper, under glass	.....	2, 1, 1 $\frac{1}{2}$ , 2, 2 $\frac{1}{2}$ , 3 (hrs.)
November 11, 1905....	101	" " " "	2, 1, 1 $\frac{1}{2}$	2, 2 $\frac{1}{2}$ , 3, 4 (hrs.)
" 30, 1905....	101	+moisture, on paper, under glass	10, 20	30, 45, 60 (min.)
December 24, 1905....	101	On paper, under glass	.....	10, 20, 30, 45, 60 (min.)
" 26, 1905....	101	" " " "	$\frac{1}{2}$	1, 2, 3 (hrs.)
" 26, 1905....	101	" " " "	.....	10, 20, 30, 45, 60 (min.)
" 26, 1905....	102	" " " "	10	20, 30, 45, 60 (min.)
" 26, 1905....	110	" " " "	.....	10, 20, 30, 45, 60 (min.)
" 26, 1905....	113	" " " "	10	20, 30, 45, 60 (min.)
April 4, 1906....	101	" " direct	5, 10, 15	20 (min.)
" 11, 1906....	102	" " " "	.....	5, 10, 15, 20 (min.)

In the work prior to December, the data showing greatest longevity were obtained, as previously noted, by rubbing the pure culture upon the paper and then making the exposure; the December data were secured from aqueous suspensions inoculated upon paper, while those in April and later were films from suspensions, dried on paper and exposed to the sun directly, i. e., without glass or other intervening medium.

Leaving out of account the earlier results, as not being satisfactory, it appears that the method of direct exposure gives as consistent and similar results with tubercle as with other non-spore-bearing bacteria; it is more sensitive than some, but less so than *B. coli*, for example. Too close comparisons are not admissible here, for the time is so short that slight but unavoidable variations may place the result on one side or the other. Apparently tubercle is not especially sensitive to light. This may be only apparently so, for, as was mentioned earlier, homogeneous suspensions are not so readily obtained with tubercle as with other bacteria, and, as a result, clumping, with consequent protection, takes place.

In this connection, another experiment will be of interest. A sample of sputum, containing numerous tubercle bacilli and also cocci, etc., was spread in thin films on papers and exposed under glass in the usual way. In the cultures where development took place, *M. pyogenes aureus* and *albus* were present; but in those that remained free from contamination, tubercle failed to develop. The periods of exposure were 10, 20, 30, 45, and 90 minutes. A repetition of this experiment gave the same results. This would seem to indicate that the tubercle bacillus is not more resistant to the action of light than were the other bacteria that chanced to be present in the sputa.

The details of an experiment made on April 4 will be interesting and instructive, especially in helping to explain the variable results obtained with tubercle, and also its apparent endurance. A watery suspension of a culture of tubercle No. 101 was employed. This suspension was filtered through a layer of glass wool, and subsequently inoculated upon paper strips. These strips were sealed at one end to the bottom of a petri dish by means of hot paraffin. After inoculation, they were exposed, uncovered, to direct bright sunlight between 12:28 and 12:54 P. M. The results are given in Table 17.

It is seen that none of the five-minute exposures was killed; that two out of four of the 10-minute exposures grew, one showing only a single colony; also two from the 15-minute lot showed each a single colony; and that none of the 20-minute cultures grew. Two controls gave excellent growth. Although the suspension from which these inoculations were made was filtered, and a macroscopic exami-

TABLE 17.  
RESULTS WITH TUBERCLE, BY DIRECT EXPOSURE

Trail No.	Time of Exposure	Result
1.....	5 min.	Good growth
2.....	5 "	Four colonies
3.....	5 "	Fair growth
4.....	5 "	Slight growth
5.....	10 "	No growth
6.....	10 "	One small colony
7.....	10 "	No growth
8.....	10 "	Good growth
9.....	15 "	Contaminated
10.....	15 "	One colony
11.....	15 "	" "
12.....	15 "	No growth
13.....	20 "	" "
14.....	20 "	" "
15.....	20 "	" "
16.....	20 "	" "
17.....	Control	Good growth
18.....	"	" "

nation showed decided improvement in homogeneity as compared with the original, yet a microscopic examination still revealed occasional clumps of bacilli aggregating 10-50 in number. Undoubtedly it is due to this tendency to clump that tubercle gives such varying results, and a false impression is obtained in regard to its true resistance to sunlight, which is probably not greater than that of the common pus cocci.

An experiment was made with Möller's grass bacillus, which is acid-fast and simulates tubercle very closely, except that it grows at room temperature on common media. In this case it was not killed in 15 minutes, on May 11, at midday. Here clumping was marked, and was accompanied by a corresponding longer period of resistance to sunlight.

It was stated by Koch that cultures of tubercle are killed in five to seven days in diffuse light. This point was also tested, cultures on paper slips being exposed for comparison. The exposures were made directly in front of a north window in the laboratory next to the window-sill, all the cultures being under glass.

In this series the pure culture was rubbed onto the paper slips. The sunlight afforded by a number of days was necessary for these long exposures. While the results are too few for drawing any positive conclusions, they do not indicate any extraordinary sensitiveness to diffuse light on the part of tubercle, for the culture of October 21 required six days of exposure and then was not killed. The cultures



TABLE 18.

DATE	CULTURE	METHOD OF EXPOSURE	RESULTS OF EXPOSURE (HOURS)	
			Growth	No Growth
September 7, 1905.....	No. 101	On egg medium	2, 5, 9 $\frac{1}{2}$ , 15, 22 $\frac{1}{2}$ , 30	.....
" 7, 1905.....	" 101	On paper+moisture	2, 5, 9 $\frac{1}{2}$ , 15, 22 $\frac{1}{2}$ , 30	.....
October 21, 1905.....	" 101	On egg medium	25, 30, 35, 40, 45, 50	.....
" 21, 1905.....	" 101	On paper	.....	25, 30, 35, 40, 45, 50
" 21, 1905.....	" 101	On paper+moisture	25	30, 35, 40, 45, 50
" 21, 1905.....	" 110	On paper	25	30, 35, 40, 45, 50

on paper slips show that diffuse light does have an effect; this is not due to desiccation, for when moisture was added the result was similar.

Taking the results for tubercle as a whole, there is no good reason to ascribe to this organism any special powers in the way of resistance or lack of resistance to sunlight; in fact, it possesses about the same order of resistance as the other nonsporogenous pathogenic bacteria.

A number of experiments were carried out to determine which rays acted bactericidally upon *B. tuberculosis*. The method adopted was to employ the paper slip cultures under a single pane of red, green, or blue glass. These glass panes were found, however, not to give pure monochromatic light, for the red filtered through the blue, and the blue through the red, while both filtered through the green. Under these circumstances, Tubercle No. 101 gave the following results:

TABLE 19.

TRIAL	DATE	METHOD OF EXPOSURE	RESULT OF EXPOSURE (MIN.)	
			Growth	No Growth
(a).....	May 3	Under colorless glass	.....	5, 10, 15, 20, 30, 45
(b).....	" 3	" red "	5, 10, 15, 20	30, 45
(c).....	" 3	" green "	5, 10, 15, 20, 30	45
(d).....	" 3	" blue "	5	10, 15, 20, 30, 45
(e).....	" 17	" colorless "	5	10, 20, 30
(f).....	" 17	" red "	5, 10	30
(g).....	" 17	" green "	5, 10, 20, 30	.....
(h).....	" 17	" blue "	5, 10	20, 30

These experiments indicate that it is the violet end of the spectrum that is fatal for tubercle, just as for other forms.

Since the data which have been recorded in the foregoing tables are distributed throughout the paper, it appears wise to collect them in a single table for convenience of reference and comparison. The table is as follows:

TABLE 20.

SUMMARY OF RESULTS OF EXPOSING BACTERIA TO DIRECT SUNLIGHT

Date	Organism	Method of Exposure	Limits of Life (Min.)
March 20.....	<i>B. coli</i>	Direct	0 and 2
" 23.....	"	"	2 " 4
" 23.....	"	"	1 " 2
April 7.....	"	"	2 " 5
" 11.....	"	"	0 " 2
May 11.....	" (No. 547)	"	2 " 5
" 11.....	" (No. 711)	"	2 " 5
" 11.....	"	"	2 " 5
April 27.....	<i>B. typhosus</i>	"	5 " 10
May 10.....	"	"	0 " 2
" 15.....	"	"	2 " 5
April 22.....	<i>B. dysenteriae</i>	"	0 " 2
May 9.....	"	"	0 " 2
" 10.....	"	"	2 " 5
April 11.....	<i>S. cholerae-asiaticae</i>	"	0 " 2
" 27.....	"	"	0 " 2
May 10.....	"	"	2 " 6
March 31.....	<i>B. diphtheriae</i>	"	5 " 10
April 27.....	"	"	0 " 2
May 10.....	"	"	2 " 5
April 2.....	<i>M. pyog. aureus</i>	"	10 " 15
" 22.....	"	"	5 " 10
May 15.....	"	"	5 " 10
April 7.....	<i>M. tetragenus</i>	"	2 " 5
" 11.....	"	"	2 " 5
" 22.....	"	"	2 " 5
May 10.....	"	"	0 " 2
April 27.....	<i>B. prodigiosus</i>	"	0 " 2
May 11.....	"	"	2 " 5
April 7.....	<i>B. pyocyaneus</i>	"	0 " 2
" 11.....	"	"	2 " 5
May 9.....	"	"	0 " 2
June 30.....	"	"	2 " 4
April 2.....	<i>Sar. aurantica</i>	"	25 " ..
May 15.....	"	"	20 " ..
" 10.....	"	Under glass	60 " ..
" 28.....	"	Direct	60 " 90
" 15.....	Pink air-coccus	"	20 " ..
" 19.....	"	Under glass	60 " ..
" 28.....	"	Direct	90 " 120
" 12.....	Möller's grass bacillus	"	15 " ..
December 24...	Tubercle 101 (human)	Under glass	0 " 10
" 26...	"	"	30 " 60
" 26...	"	"	0 " 10
April 4.....	" 101	Direct	15 " 20
December 26...	" 102 (human )	Under glass	10 " 20
April 11.....	" 102 (human)	Direct	0 " 5
December 26...	" 110 (bovine)	Under glass	0 " 10
" 26...	" 113 (avian)	"	10 " 20
April 23.....	" 113	Direct	15 " ..

## PRACTICAL BEARINGS OF THE RESULTS.

In reading the publications of the workers in this field, especially those of the earlier investigators, one notices that they expected rather more disinfectant action from the sunlight than was found; and so there seems to be something of a tone of disappointment. However this may be, it is certain that the results they obtained fell far short of doing justice to the disinfecting action of the sun's rays. While this action has been considered important from a sanitary view-

point, it is certain that it is much more powerful than previous results have indicated. Were it not for this powerful repressive agent, coupled especially with the repressive action of desiccation, it seems, indeed, that our chances for waging a successful war against disease and other objectionable bacteria would be slight; but when we realize that 2 to 10 minutes of active sunlight are sufficient to kill them when directly exposed, we can readily understand how the vast majority of all such bacteria are effectively destroyed, and only an infinitesimal number remains. This gives sanitary science new hopes and fresh courage with prospects of the highest success.

These results explain, in a measure, the advantages of a dry climate, such as the western and southwestern portions of the United States possess, where, due to the dryness and the superabundant sunshine, most bacteria, and especially the non-spore-bearing disease germs are quickly destroyed. Above all, they emphasize the importance of well-lighted and ventilated houses. The sunlight is a friend and protector of our welfare and should not be barred from our homes by shutters and heavy shades; for there is truth in the Italian saying: "Where sunlight enters not, there the physician goes."

#### CONCLUSIONS.

The conclusions arrived at may then be summarized as follows: The methods heretofore employed in testing the bactericidal action of sunlight do not seem to be well suited for this determination, since the results do not indicate the full power of this agent.

The light is absorbed by the medium in which the bacteria are planted, and the glass cover both absorbs and reflects a considerable portion of the effective rays. A more suitable method consists in planting the bacteria upon glass or paper and exposing directly, i. e., without glass or other cover, to the sun's rays. By this method most of the non-spore-bearing bacteria, including *B. tuberculosis*, *B. diphtheriae*, *B. typhosus*, *S. cholerae-asiaticae*, *B. coli*, *B. prodigiosus*, and others, are killed in a remarkably short period of time, varying from 2 to 10 minutes. This time is considerably lengthened if the suspension used is not homogeneous and the bacteria consequently become clumped or bunched in the film. Certain saprophytic bac-

PLATE 2.







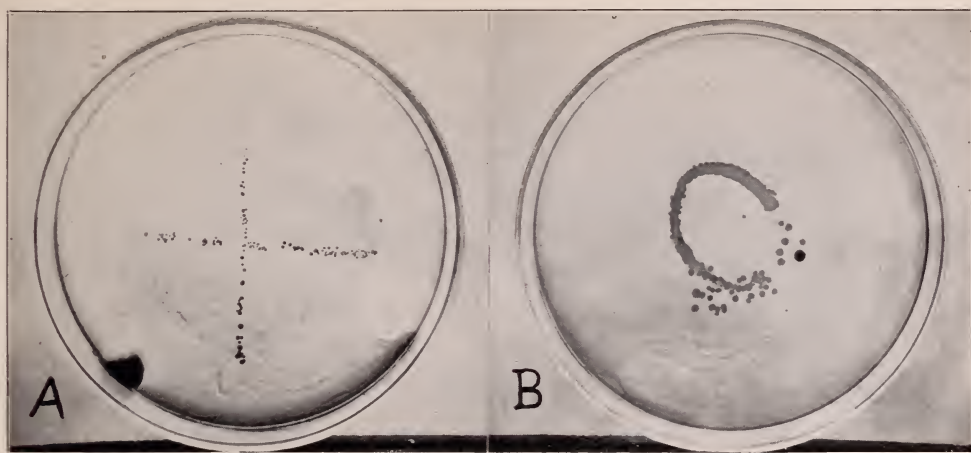


FIG. 1.



FIG. 2.



teria, such as the micrococci found in the air, require a much longer period of exposure to kill them, the time being upward of an hour.

By the method employed in the foregoing work, practically all the disturbing and complicating factors of the older methods are eliminated: the time required for exposure is so short that desiccation becomes a negligible factor, or practically so; the formation of bactericidal by-products in the medium, such as hydrogen peroxide, phenol, organic peroxides, etc., is entirely removed, and consequently the action must be ascribed wholly to the sunlight.

So far as *B. tuberculosis* is concerned, it is the more refrangible rays, or the violet end of the spectrum, which are bactericidal for this organism.

Finally, the results by direct exposure of the bacteria indicate that sunlight is a much more powerful germicidal agent, and consequently a more important hygienic factor, than it has heretofore been considered; that the bacteria, when freely exposed, are killed in one-fifth to one-twentieth of the time formerly considered necessary.

#### ACKNOWLEDGMENTS.

In closing I wish to express my deepest gratitude to Professor H. L. Russell for suggesting and directing the present investigation; I have also to acknowledge with thanks the kindly assistance of Professors W. D. Frost and E. G. Hastings, and Dr. C. A. Fuller, all of whom have been ever ready to promote and encourage the work.

#### EXPLANATION OF PLATES.

##### PLATE 2.

Tubercle cultures grown on egg medium after exposing to direct sunlight as follows: A— $\frac{1}{2}$  hr.; B—1 hr.; C— $1\frac{1}{2}$  hrs.; D—2 hrs. (no growth). Note the area of the infected paper in each tube.

##### PLATE 3.

FIG. 1.—Showing petri plates exposed uncovered to direct sunlight. A is *M. tetragenus* exposed 2 minutes; B is a pink micrococcus from the air, and was exposed 60 minutes. The cross and circle are made up of the bacterial colonies; they serve as mechanical devices for distinguishing the culture inoculated from the foreign or contaminating bacteria.

FIG. 2.—*B. coli* in agar plates exposed to sunlight. A=control; B=10 min; C=20 min. D=30 min.; E=45 min.



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## HISTORICAL STUDY OF LEGISLATION REGARDING PUBLIC HEALTH IN THE STATES OF NEW YORK AND MASSACHUSETTS.\*

SUSAN WADE PEABODY.

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### INTRODUCTION.

FOR the purposes of the present paper it has seemed best to limit the discussion to the legislation of two states, New York and Massachusetts, taken as leading types. These states were chosen because they were among the first to have an established government and because in the enactment of laws of all sorts other states have generally followed the lead of one or the other of these two. In connection with health legislation several instances have been discovered in which the laws of New York and Massachusetts are copied verbatim by other states.

It has also seemed best to consider health in the limited sense of being opposed to disease instead of in the wider sense which would include all hygiene and sanitation. Health legislation, taken in this sense, would not include laws which were primarily hygienic, economic, or professional in their bearing, such as laws for pure foods and drugs, for pure and plentiful supplies of water and ice, for the construction of sewage and drainage systems, for proper dwellings, for diseases of animals, for the regulation of trades and professions, for the erection and maintenance of public hospitals, and for the collection and preservation of statistics. In the sense in which health has been used

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health legislation would include all laws which attempted to control the spread of disease by direct means, as opposed to those which sought the same end by indirect means.

The material for the paper has been arranged in two parts: in the first an attempt has been made to show the causes of legislative action so far as they could be ascertained, and to state the substance of the laws both as reflecting the progress of scientific knowledge and as showing the increased powers of control given to administrative authorities; in the second part a comparative study has been made of the administration of the laws as provided in the laws themselves, both as to the administrative authorities and as to the means of enforcement.

It has been easy to find the statutes, more difficult to find the reasons for their enactment, and most difficult to find historical material in regard to the administration of the laws. Reports of boards of health are taken up so exclusively with results of investigations and statistics that they give little space to the consideration of the workings of the laws or the details of administration. It has taken long and diligent search through many volumes devoted to the scientific side of the question to unearth the facts contained in the paper.

The legislation of the past has been instigated chiefly by great epidemics of a few specific diseases: yellow fever, smallpox, and cholera. Under a wise administration of the present laws guided by modern scientific knowledge such epidemics may be considered as things of the past; in the fight of the state for public health those diseases have been conquered. The attention of health authorities at the present time is largely engrossed with the prevention of diseases of another class, more insidious if less spectacular in their ravages: such are tuberculosis, diphtheria, pneumonia, and cancer. With further advances in scientific knowledge we may have legislation to prevent the spread of these diseases, as of those of the past, by compulsory measures. The sanctions of the early laws were vigorous, their enforcement often drastic; modern methods lean more toward education and persuasion; yet the time may come when it will be made a crime to aid in disseminating any form of disease germ. One Louisiana statute, that of 1886, chap. 120, looked strongly in that direction with regard to the germs of yellow fever. And such legislation will be upheld by the courts as consistently as has been that of the past.

## PART I. HISTORY OF HEALTH LEGISLATION.

### CHAPTER I.

#### LEGISLATION IN NEW YORK.

LEGISLATION in New York in regard to health matters shows three distinct phases of growth, each occupying a period, roughly, of fifty years. The legislation of the first of these periods consists almost entirely of attempts to exclude contagious disease coming from external sources, particularly yellow fever from the West Indies, by means of quarantine applied at the port of New York; the second period shows the development of the idea of local control of local health matters by local authorities, especially in regard to nuisances, beginning with a few of the larger cities and finally extending to all cities and villages within the state; the third period is marked by provision for scientific investigation and by the growth of centralization and increased efficiency of administrative machinery.

#### A. FIRST PERIOD, 1755-1805.

New York, with her magnificent harbor, has always been the main natural gateway of commerce for the North American continent. From the days of the first settlement ships from all parts of the world have sought her trade. It is difficult to determine at what time the colonists, finding that smallpox, ship or typhus fever, and yellow fever were as likely to be brought in such vessels as silks and spices, first attempted to prevent their introduction by quarantine, although there is reason to believe that there was at least one such attempt under the Dutch rule in 1647.

After the English occupation, for more than 75 years, quarantine seems to have been enforced occasionally without special legislation by order of the governor and council with no other authority than the general powers of government conferred by the crown. Such instances occurred in 1702, 1714, 1716, 1725, 1738, and 1742;<sup>1</sup> and also by order of the city council as early as 1689.<sup>2</sup>

<sup>1</sup> *Memorial History of N. Y.*, 2, p. 168; *Minutes Colonial Council*, 17, p. 271; 19, pp. 147, 148; 21, p. 29; *Minutes Common Council*, 4, p. 429; *Report N. Y. State Bd. H.*, 3, p. 127. For complete titles of books of reference see Bibliography, p. 140.)

<sup>2</sup> *Min. Com. Coun.*, 1, pp. 208, 209.



Various epidemics visited the colony during the seventeenth century, smallpox was of frequent occurrence and was considered a more or less necessary evil;<sup>3</sup> the "fatal epidemic," which appeared in September, 1668, in New York City, is supposed to have been yellow fever, as also that which broke out in Philadelphia in 1672; although the first authentic yellow fever, recognized at the time as such, was that which raged in Charleston and in Philadelphia in 1699. In the latter city the disease, known as the "Barbadoes distemper," lasted from early August until late October, and carried off in that time 220 of the inhabitants. It was believed to have been brought from the West Indies and was doubtless the immediate occasion of the first quarantine law found on the statute books of any of the colonies—that of the colony of Pennsylvania of 1700—and was also doubtless the cause of the attempted law of the same year which was passed by the provincial assembly of Massachusetts.<sup>4</sup> From this time and throughout the eighteenth century there were frequent epidemics of yellow fever; in New York City alone it occurred in epidemic form no less than 10 times before 1800; i. e., in 1702, 1743, 1745, 1751, 1762, 1791, 1793, 1795, 1797, 1798.<sup>5</sup> The epidemic of 1702 was known as "the great sickness," but although the assembly was obliged to move over to Jamaica, L. I., there seems to have been no legislation in regard to health;<sup>6</sup> the city council was roused, however, to pass an ordinance for cleansing the streets.<sup>7</sup>

The earliest action by the legislature of New York regarding health was the quarantine law of 1755. By this law it was enacted that all vessels having smallpox, yellow fever, or other contagious distemper on board, and all persons, goods, and merchandises coming or imported in such vessels, and all vessels coming from places infected with such distempers should not "come into any of the ports or harbours of this city or nearer the same than Bedloe's Island" and "shall be obliged to make their quarantine there or in such other place for such time and in such manner as the Governor or Commander-in-

<sup>3</sup> In 1721 the Colonial Council decided that no quarantine against Boston was necessary since it was only smallpox which was raging there! *Min. Col. Coun.*, 13, p. 200.

<sup>4</sup> *Infra*, p. 42.

<sup>5</sup> Webster, *History Epidemic Diseases*, 1709; Griscom, *History Yellow Fever in New York*, 1808; *Report Com. N. Y. House on Quarantine*, 1846; *Report N. Y. State Board of Health*, 2, p. 234.

<sup>6</sup> Webster, *loc. cit.*, 1, p. 216.

<sup>7</sup> *Min. Com. Coun.*, 2, pp. 195-97; *Mem. Hist. New York*, 4, p. 505; *infra*, p. 77.

Chief, by and with the advice of His Majestie's council, shall think proper and reasonable to direct and appoint, and . . . until discharged from quarantine, no such person or goods shall come on shore or be unloaded, on any other vessel in this colony or in New Jersey, without a license of the Governor and Council." A fine of £500 was imposed for violation of orders, and persons coming on shore might be returned to their vessels by force or isolated. Pilots were to hail incoming vessels and give notice of the law, with a fine for neglect to do so. A false report of the captain was also punished by fine. The governor was to appoint a surgeon or physician as inspector. A few months later (September 11, 1755) a supplementary act was passed explaining the provisions of the first act as to isolation of those infected and extending the act to all infected persons not "belonging to the vessel."

This early law was re-enacted from time to time with slight variations, but until 1784 always for a limited period. Other health matters in addition to quarantine for the port of New York were frequently included; such were the provisions for lazarettos and pest-houses in the laws of 1758 and 1778 and those concerned with inoculation in 1763 and 1778.<sup>8</sup>

In 1784 the quarantine law was passed without a time limit, but the provisions were essentially the same. Indeed, there was no important change in the substance of the earlier laws until the dreadful devastation of Philadelphia by yellow fever in 1793 stirred the whole country. At that time there were more than 4,000 deaths in a population of less than 60,000. All who could fled to surrounding places; from many the fugitives were turned back toward the plague-stricken city. The greatest alarm was felt in New Jersey, Maryland, and New York; stage lines were forbidden to carry passengers from Philadelphia; panic was general. The alarm in New York was first officially announced by a letter from the mayor to the physicians of the city, dated September 11, 1793, in which he requested them to report to him in writing the names of all persons who had arrived from Philadelphia sick of a contagious disease that they might be removed out of the city.

"In this letter the mayor declared his opinion clearly that the intercourse with Philadelphia could not be lawfully interrupted by any power in the state. The 12th

<sup>8</sup> *Infra*, pp. 38, 47.

appeared a proclamation from Gov. Clinton, which, referring to the act "To Prevent the Bringing-in of Infectious Diseases," prohibited, by the terms of that act, all vessels from Philadelphia to approach nearer to the city of New York than Bedloe's Island . . . till discharged. The silence of this proclamation respecting passengers by land seemed to imply that the governor's opinion on the subject was the same as that of the mayor.<sup>9</sup>

At the next session of the legislature this defect in the law was remedied by an amendment<sup>10</sup> giving the governor power to proclaim quarantine against vessels arriving from "all ports whatsoever" and against persons arriving from infected places by land as well as by water. The law was also extended to include the cities of Albany and Hudson, and required the appointment by the governor of a physician as health officer for each of these cities as well as for New York City. In the absence of the governor, the mayors of the three cities were to execute the act, and two or more justices of the peace of any town lying on the Hudson River. The same persons and two or more justices of any inland town were authorized to detain, examine, isolate, or send out of the state any person coming by land who was suspected of an infectious disease.

Following the great epidemic in Philadelphia in 1793 and rivaling it in virulence, there raged a conflict among the physicians of that city as to the cause of the disease. One party, headed by Dr. Rush, and including many eminent members of the Academy of Medicine, held to the opinion that the disease was of domestic origin, arising from atmospheric conditions acting upon putrid animal and vegetable matter. This party demanded cleaner streets, better drainage, the filling of low lots and filthy places. The other party, backed by the College of Physicians, was equally certain that the fever was imported from the West Indies or southern ports and clamored for stricter quarantine laws. The controversy extended to New York and both sides found adherents among the physicians and people of that city. In spite of the improvements in the quarantine laws, there had been a few cases of yellow fever in New York in 1794, but the summer of 1795 brought a serious epidemic with 750 deaths. Bitter discussions arose, as earlier in Philadelphia, as to the cause of the epidemic and the best means of preventing future visitations. The origin was laid to putrid hides stored in a damp cellar, to stagnant

<sup>9</sup> Carey, *Short Acct. Malignant Fever Lately Prevalent in Philadelphia*, 1793, p. 39.

<sup>10</sup> 1794, ch. 53.

water on low or newly made land, to decayed coffee brought from the West Indies; on the other side, it was traced more or less directly to cases of yellow fever arriving on vessels from infected ports. Quite likely the fact that the first victim was Dr. Treat, the health officer of the port, strengthened the cause of those who believed that the disease was of foreign origin.<sup>11</sup>

Both theories found expression in the important health law passed by the legislature at its next session, in 1796.<sup>12</sup> This law included both quarantine and, for the first time, nuisances. Under its terms certain vessels from foreign ports were to perform quarantine as of course; such were: those containing more than 40 passengers; those arriving with a case of fever on board; those on which a death from fever had occurred during the voyage; and those which came from infected places. The governor was given power to add to the list of places from which vessels must perform quarantine, and to proclaim quarantine against places in the United States, prohibiting intercourse by land or ferries. Physicians were required to report cases of infectious diseases. On the other side of the law, urged by the non-contagionists, the mayor and council were given authority to make by-laws, rules, ordinances, and orders for filling low lots, cleansing streets, yards, cellars, and vaults, for regulating noxious trades, and for removing and destroying all offensive and putrid articles and substances; but such ordinances were not to exist more than twelve months unless confirmed by the governor. The execution of the law was placed in the hands of a physician-health officer, and "several" commissioners of health. The commissioners were empowered to order to quarantine any vessel which had been brought to anchor elsewhere, and any vessel from the wharves on which was a case of infectious disease, or goods "apprehended to contain infection."

The law of 1796 was a great step in advance, but in spite of all precautions there were some cases of yellow fever in the summer of 1796. An amendment to the law early in 1797<sup>13</sup> subjected coasting-vessels from south of Cape May to quarantine as of course; prohibited noxious trades within certain limits; fixed the number of health commissioners besides the health officer at three, and gave them

<sup>11</sup> Five of the first seven health officers of the port of New York died of yellow fever (Townsend, p. 232).

<sup>12</sup> 1796, ch. 38.

<sup>13</sup> 1797, ch. 16.



power to make and execute rules and orders for the abatement of nuisances covering the same ground as the ordinances which the mayor and council had been authorized to make by the law of 1796. The law required these rules to be reported to the governor, by whom they might at any time be suspended or repealed. After the first of the following July the noxious trades, i. e., the manufacture of glue, soap, candles, and the dressing of skins, were entirely prohibited within certain sections of the city, the mayor and council to treat with the owners for the expenses of removal, with provision for a jury to fix the compensation in case no agreement could be reached. The effectiveness of this section of the law was lessened, however, by an amendment within two months (March 28, 1797) empowering the governor and two commissioners to grant permits for the manufacture of soap and candles within the boundaries indicated.

The fever appeared again in the summer of 1797. In the following winter, 1798,<sup>14</sup> all previous laws were repealed and a new law passed. The quarantine provisions of this act were not greatly changed from the earlier laws; the health officer was given somewhat wider powers of search and was required to report to the health office the name of each vessel found, in his judgment, free from infection that further directions might be given concerning her procedure and the disposal of her cargo, and all coasting-vessels were made subject to examination; but the provisions as to nuisance were fuller and the powers of abatement given to the health commissioners more ample. They were required to make "strict examination" into nuisances, and, if found injurious to health, they might issue orders for abatement. In case such orders were not complied with, the nuisance might be abated by the sheriff at their request and the owner punished for misdemeanor.<sup>15</sup>

In this law there was a strenuous endeavor to protect the health of the people by all the means suggested by adherents of both parties, and yet the summer of 1798 saw the most widespread visitation of yellow fever the country had known; it extended as far north as Portsmouth, N. H., and included Boston and several Connecticut towns as well as Philadelphia and New York in its ravages; in the last-named city there were 2,500 deaths. The whole country

<sup>14</sup> 1798, ch. 65.

<sup>15</sup> See later chapter on "Enforcement," p. 85.



was aroused and the question of a national quarantine was brought before Congress by a message from the President.

In New York, by an amendment to the existing law,<sup>16</sup> the Marine Hospital on Staten Island was provided for the detention of diseased persons instead of the lazaretto, and the quarantine anchorage, formerly at Bedloe's Island, was fixed as near the hospital as possible; no vessels were to pass quarantine without a permit from the health officer; for the first time vessels were classified according to the port of departure, and those only made subject to quarantine as of course which came during the summer months (May 31–October 31) from tropical ports; all others, including coasting-vessels from the south of Sandy Hook, were subject to examination, with detention and purification at the discretion of the health officer; communication with quarantined vessels or the Marine Hospital was forbidden; quarantined vessels must be indicated by appropriate colors; and lodging-house keepers must report within twelve hours a case of sickness of a "seafaring man or sojourner," all enforced with appropriate sanctions.

During the same winter certain local health matters in New York City were for the first time made the subject of a separate law.<sup>17</sup> In view of the discussions among physicians and others as to the origin of yellow fever, the preamble of this act "To Invest the Mayor and Aldermen with Adequate Powers" is of especial interest:

WHEREAS, The general health of the state is connected with the health and safety of the city of New York, which has lately been visited by a destructive and epidemic disease, the causes of which, as far as human wisdom can discover them, ought to be removed and such measures adopted as by divine favor may prevent the return of that fatal malady, and

WHEREAS, It is represented to the legislature that noxious exhalations from sunken and damp lots of ground, deep, damp cellars, narrow and confined streets, foul and ill-constructed sinks and privies, unfinished water lots, foul slips, putrid and unsound provisions, and other evils of a similar nature, together with the practice of interring the dead in the heat of summer in improper places, and without due precautions, are among the natural causes to which the existence and malignity of that disease may be in a great measure, attributed: Therefore . . . .

it was enacted that the mayor and council should have power to pass by-laws and ordinances for filling up or draining low lots or cellars, for filling or mending public slips, private sinks or privies, for directing

<sup>16</sup> 1799, ch. 19.

<sup>17</sup> 1799, ch. 70.

the mode of construction of vaults or requiring subterraneous drains, for regulating the interment of the dead, for regulating boarding-houses and taverns, and for the destruction of unsound provisions or other substances. They might also appoint one or more inspectors of lots to execute the law. Other classes of nuisances, included in the law of 1796,<sup>18</sup> were not included in the powers bestowed upon the mayor and council, and continued to be regulated by the rules and orders of the commissioners of health.

That this law of 1799 was considered something of an emergency measure would appear from the fact that it was limited to three years. At the end of that time, however, it was re-enacted for another three years, with the addition of powers for the regulation and license of pawn-brokers and dealers in second-hand articles; the taxation and destruction of dogs; for requiring from physicians opinions with regard to the health of the city, and certificates of death stating the nature of the disease, without which there could be no burial; and for the regulation or prevention of interments within the city. At the expiration of the three-year limit the law was made permanent by an amendment to the city charter in 1806.<sup>19</sup>

There seems to have been no serious outbreak of yellow fever in New York between 1798 and 1803. During that time the quarantine law was amended<sup>20</sup> by increasing the power of the health officer so that he might require the unloading and purification of any vessel from a port infected with pestilential fever, or on which sickness or death from such cause had occurred during the voyage; it gave him wide discretionary powers over the cleansing or destruction of wearing apparel or bedding; it also forbade the importation during the summer months of cotton, hides, damaged coffee, or peltry on pain of forfeiture.

In 1801<sup>21</sup> a new law was passed putting in better form the quarantine provisions of the earlier laws for the port of New York but adding nothing of much importance. Quarantine for the cities of Albany and Hudson continued to be instituted by the governor (in his absence by the mayor or recorder) and to be performed for such time and in such manner as directed by him; for other ports, by two justices of the peace.<sup>22</sup> To the powers of justices for inland towns was added

<sup>18</sup> Repealed by 1798, ch. 65.

<sup>19</sup> 1806, ch. 126.

<sup>20</sup> 1800, ch. 120.

<sup>21</sup> 1801, ch. 86.

<sup>22</sup> 1804, ch. 79; *supra*, p. 6.

the appointment of such persons as they deemed proper to aid in the execution of their powers.

In 1803 there was another visitation of yellow fever "appalling" in character, over 600 deaths occurred, chiefly along the wharves; in 1804 more than 700 died; in 1805, 270; all this, it must be remembered, in a city of 30,000, a city the size of Elgin, Ill., or Newport, R. I.

Several supplementary quarantine laws were passed during this time. That of 1804<sup>23</sup> forbade vessels from infected ports, or with disease on board, to approach nearer the city than quarantine grounds at any time between June 1 and October 1, unless bound to a port outside the state in Long Island Sound; no persons or goods from such a vessel might approach the city without a written permit from the health officer; no vessels from the West Indies, South America, the United States south of Savannah, Ga., or from the Mississippi River, the Mediterranean, Africa, or Asia (except Canton and Calcutta) should approach, after examination and a permit by the health officer, nearer than 300 yards to the city, but must discharge their cargoes by lighters subject to the regulations of the commissioners of health, and be cleansed and disinfected; and<sup>24</sup> no permit was to be granted by the health officer for a nearer approach. This section, forbidding an approach nearer than 300 yards, was considered by the health officials of ensuing years the most important provision of the health laws, and to it they attributed the comparative immunity of New York City in the years which followed.<sup>25</sup>

In 1806<sup>26</sup> all vessels arriving during the summer months from the West Indies or Mississippi River were required to remain at quarantine ground at least four days, or longer at the discretion of the health officer, with no communication with the city except as permitted, whether they were from infected or healthy ports, and whether or not they had any case of disease on board.

By the enactment of these laws "the quarantine establishment had become reduced to a regular system, and endowed with vigorous and efficient powers."<sup>27</sup>

Briefly reviewing the progress of health legislation of New York

<sup>23</sup> 1804, ch. 8.

<sup>24</sup> 1805, ch. 31, sec. 3.

<sup>25</sup> *Proceedings and Debates Third Nat. Quarantine and Sanit. Convent.*, New York, 1859.

<sup>26</sup> 1806, ch. 79.

<sup>27</sup> P. v. Roff, 3 Park 216 (1856).

during the half-century from 1755 to 1806, we find a series of twenty or more laws, in almost every case the direct result of an epidemic of yellow fever, largely attempting to preserve the health of the state from invasions of disease from external sources through the port of New York City by means of quarantine. The law grew from a simple grant of power to the governor to proclaim quarantine and detain infected vessels to a detailed law creating a complete system, and placing great power in the hands of the officers appointed to enforce it. Throughout the whole series the execution of the law was by appointees of the governor: from 1755 to 1796 a physician-inspector; from 1796 a health officer and board of health commissioners.

During the latter part of this period we find the beginnings of the tendency toward control of local health matters by local authorities for the prevention of the spread of disease which marks health legislation during the next half-century. The first indication of this tendency was the provision for pest-houses in the law of 1778. After the yellow fever epidemic in 1795 there was a temporary grant of power to the mayor and council to make by-laws and ordinances for the cleansing of the city and the abatement of nuisances, but within a short time the power was given to the commissioners of health, who were state officers, and in both cases rules and regulations could not survive without the approval of the governor.

#### B. SECOND PERIOD, 1805-1855.

##### 1. *The Growth of Control by Local Authorities over Nuisances.*

During the next period of fifty years health legislation in New York was concerned largely with the development of the control over local conditions by local authorities. The fight against yellow fever had been successfully waged and the epidemics which occurred during this period were due rather to inefficient administration than to serious defects in the laws. But during this period (especially during the latter part) a new enemy appeared, Asiatic cholera, against which quarantine at the port of New York alone did not avail and in fighting which other weapons—cleanliness and better local sanitary conditions—must be used.

a) *In New York City.*—Dirt and disease had always been more or less associated in the minds of both the medical profession and the



law-makers. In colonial days the city council, under the general grant of powers contained in the early charters and even before, had passed numerous ordinances, as in 1670, directing householders to cleanse the streets.<sup>28</sup> Not until 1796, after the great epidemic of yellow fever in 1795, did the legislature grant specific authority to the mayor and council to pass ordinances covering these local conditions.<sup>29</sup>

In 1797<sup>30</sup> the commissioners of health were given power to make and execute rules and orders for the same purposes and in 1798 and again in 1801 these powers were renewed with even wider powers of abatement.<sup>31</sup>

In 1799 local matters were for the first time made the subject of a separate law, by which ordinances passed by the city council upon certain matters related to health, chiefly in regard to drainage, were to be executed by their own appointees.

The act of March 9, 1805,<sup>32</sup> transferred to the mayor and council of the city the powers, previously granted to the commissioners of health in 1797, 1798, and 1801, to make and execute rules and orders for cleansing streets, vaults, and other places, the control of noxious trades, the removal of infected persons and offensive or tainted substances, and the general abatement of nuisances. They were empowered to institute from time to time a board of health, consisting of the commissioners of the health office, and such other persons as they thought proper, and to invest this board with necessary powers. This act of 1805 marks the beginning of a new era, and is important in two directions: it took from the commissioners of health, who were state officials, all power of making rules for the government of New York City in health matters and placed it in the hands of the regularly constituted authorities, and it enabled these city authorities for the first time to establish a board of health with powers for the enforcement of the city health ordinances, although, since the commissioners were *ex-officio* members of the board, and the mayor was appointed by the governor the city was not yet entirely divested of state control.

<sup>28</sup> *Min. Com. Council*, 1, p. 7; also 1, pp. 13, 28, 36, 167, 219, 245, 224, 247, 237, 376, 392; 2, pp. 21, 74, 183, 95, 141, 195, 246, etc.; also against nuisances, 1744, *ibid.*, 5, p. 118; and for the regulation of slaughter-houses from 1676 on, *Min. Com. Coun.*, 1 p. 46.

<sup>29</sup> 1796, ch. 38; *supra*, p. 7; *infra*, p. 78.

<sup>31</sup> 1798, ch. 65; 1801, ch. 92; *supra*, pp. 8-10.

<sup>30</sup> 1797, ch. 16; *supra*, p. 8.

<sup>32</sup> 1805, ch. 31.



The acts of 1811 and 1820<sup>33</sup> continued the board of health as organized in 1805, but the act of 1823 no longer retained the three health commissioners as *ex-officio* members.<sup>34</sup> Although these three laws were primarily quarantine laws, they contained a few items containing additional powers for the city. By the law of 1820 the mayor and council were given power to appoint health wardens and "other suitable officers" for the inspection of houses, lots, and other places; by that of 1823 the board of health were authorized to take all measures in their opinion necessary to cleanse buildings, yards, and other places, and "to do or cause to be done everything in relation thereto, which, in the opinion of the board, may be proper to preserve the health of the inhabitants."<sup>35</sup>

Already in 1823 we find grumblings over the inefficiency of the new board of health.<sup>36</sup> In 1846 a committee of the House, appointed to investigate the quarantine laws, included in its report complaints of citizens about the sanitary condition of the city—unclean streets, noisome smells, nuisances, garbage on vacant lots, slaughter-houses, and a long list of other unsanitary conditions. The committee found:

There are evils still existing and sources for feeding these diseases (cholera, smallpox, etc.) which depend upon the city authorities or its citizens, which no wise man can approve. The sanitary regulations or their practical workings, are subject to the severest censure.<sup>37</sup>

In the spring of 1849, with the immediate prospect of another epidemic of cholera, an amendment to the city charter<sup>38</sup> created an executive department with a "city inspector," elected for three years, at its head. This department was "to have cognizance of all matters relating to the public health of the city."

About the middle of May cholera broke out in the "Five Points," the most filthy and squalid part of the city, and within a month had become epidemic. The board of health appointed a committee of nine to whom the whole power of the board was trans-

<sup>33</sup> 1811, ch. 175; 1820, ch. 229.

<sup>34</sup> 1823, ch. 71.

<sup>35</sup> The reasons for not retaining the health commissioners as members of the board of health are difficult to find. It was a time, however, of much political restlessness and this may have been merely a part of the growing feeling that city affairs should be free from state supervision. The new constitution of New York in 1821, provided (Art. 4, sec. 10) that "mayors of all cities should be appointed by the common councils;" up to this time the mayor of New York had been appointed by the governor of the state.

<sup>36</sup> Townsend, *Account of Yellow Fever in N. Y. in 1822-23*, 1823.

<sup>37</sup> *Report Com. N. Y. House on Quarantine Laws*, 1846.

<sup>38</sup> 1849, ch. 187.

ferred. This committee cleansed the streets and "gave the city such a thorough purification as had never been known before;" they turned schoolhouses into hospitals over the protest of the board of education; they provided nurses for the sick, and burial for the dead; but in spite of their efforts the disease carried off more than 5,000 victims that summer. In the report of the committee, presented to the board of health at the close of their labors, they complained bitterly that "the department, having cognizance of nuisances, is wholly inadequate."<sup>39</sup>

The year 1850 is a marked one in health legislation in New York; two important laws were passed, one concerned with the health of the city and port of New York, the other with that of the rest of the state.

The first of these laws<sup>40</sup> was divided into three parts: "The Officers of Public Health in the City of New York;" "Quarantine at the Port of New York;" and "Internal Regulations for the Preservation of the Public Health in the City of New York." The second title—"Quarantine"—was a revision with important modifications of the quarantine law of 1846;<sup>41</sup> the other two parts contained a radical departure from the existing laws. The legislative powers previously vested in the board of health were transferred to the mayor and council, who, when acting in relation to the public health of the city, were to be known as the board of health, and were to hold their sessions behind closed doors; the presidents of the board of aldermen and of the board of assistant aldermen, the health officer, the resident physician, the health commissioner, and the city inspector were made "commissioners of health;" they were to meet daily with the mayor during the summer and were to render advice to the board of health and city inspector in regard to all matters connected with the health of the city. The health officer, in charge of quarantine, was still to be appointed by the governor, but the resident physician and health commissioner, whose duties were to visit the sick and perform other professional duties, as required by the board, were to be appointed by the mayor and council, as was also an "inspector of vessels;" the city inspector was elected.<sup>42</sup> Visiting, hospital, and consulting physicians were to be appointed

<sup>39</sup> *Report of Sanitary Com. to N. Y. Bd. H.*, 1849; Stillé, *Cholera*,

<sup>40</sup> 1850, ch. 275.

<sup>41</sup> *Infra*, p. 22.

<sup>42</sup> 1849, ch. 187.

by the board of health, i. e., the mayor and council, who fixed their duties and compensation. To the city inspector were given many powers formerly exercised by the board of health or the commissioners: he might appoint, with the consent of the board of aldermen, all of his subordinates, or "health wardens;" he might authorize them "to enter and examine buildings and places of every description;" he might hale before the board of health the owner of any business in his judgment detrimental to health; and he might "give all directions and adopt measures for cleansing and purifying buildings" and other places; persons "disobeying any order of the inspector, or board of health . . . to abate or remove a nuisance in the manner and at the time described in such order" were liable to arrest and punishment. The powers and duties of the board of health, now the mayor and council, in the matter of making regulations, were not greatly altered; in addition to those previously exercised, they might take possession of and occupy for temporary hospitals during any epidemic any building, whether public or private, with payment of a just compensation to the owner if a private building were taken.

b) *Legislation affecting localities outside of New York City.*—Outside of New York City local control of health matters by law was of slow growth and only by special law—included in the charters of cities and villages—until 1850. The common council of Albany had been authorized in 1806<sup>43</sup> to appoint as many of its own members as were necessary to form a board of health to assist the mayor in carrying out the provisions of the health laws; the board to enjoy like powers with the New York City Board of Health. The village of Brooklyn in 1824<sup>44</sup> and the city of Brooklyn in 1834<sup>45</sup> enjoyed the right to appoint a board of health with ample powers; the charters of the cities of Buffalo in 1832, Syracuse in 1847, and Utica in 1849 granted ample powers over health matters with provision for a board of health, as did the revision of the charter of Rochester in 1844. The three other cities of this time had no boards of health under their charters, but together with many villages incorporated under special acts had general powers over nuisances, cleaning of streets, and in some instances over slaughter-houses and noxious trades.

<sup>43</sup> 1806, ch. 109.

<sup>44</sup> 1824, ch. 241.

<sup>45</sup> 1834, ch. 92.

The only general law previous to 1850 with regard to boards of health and control over matters relating to health was that passed in 1832,<sup>46</sup> which was not a permanent act. The invasion of cholera in that year brought up a discussion among medical men, similar to that concerning yellow fever, as to the nature of the disease, whether it was due to imported contagion or to an increased virulence of the ordinary summer cholera, brought about by filth, lack of proper drainage, and unusual atmospheric conditions; and whether it was best combated by quarantine restrictions, or by increased attention to cleanliness and other sanitary conditions. The "cholera act," although it was largely a quarantine measure, included also the creation of boards of health and control over nuisances. Unfortunately, this law, although re-enacted twice, was each time limited in duration, and, covering only the years of the first cholera epidemic, 1832-36, lapsed in the latter year. Affairs then reverted to their former condition and remained quiescent until an invasion of cholera in 1848-49 again roused the community. It appeared in 1848, again brought by immigrants from Europe. In New York the disease was confined to the quarantine grounds, but in New Orleans, which at that time had no quarantine, it gained a foothold and from there spread up the Mississippi and Ohio rivers and thus over the whole middle country and with the gold-seekers even west to San Francisco. Early in 1849 it came again to New York with immigrants and this time the quarantine did not avail to keep it out. In New York City alone there were more than 5,000 deaths.<sup>47</sup> One important result of this epidemic, the passage of a new health law for the city and the port of New York, has already been noted; another important result was the revival, without a time limit, of the cholera act of 1832, which thus became the first permanent law in the state requiring the appointment of boards of health and health officers in every city and village in the state, and of health officers in all the towns. The only additional power given beyond those of 1832 was to regulate, prohibit, or prevent all communication with houses or places in which were persons who had been exposed to infectious or contagious disease.<sup>48</sup>

In consequence of another epidemic of cholera in 1866 and of judicial decisions unfavorable to the abatement of nuisances by local

<sup>46</sup> 1832, ch. 333.    <sup>47</sup> *Infra*, p. 23; Stillé, *Cholera*; Wendt, *Asiatic Cholera*.    <sup>48</sup> 1850, ch. 324.



boards,<sup>49</sup> the law was amended in 1867 to include the making and carrying-out of orders for abatement among the powers of such boards, and gave in detail the procedure to be followed in such cases. There were no other amendments until after the establishment of the state board of health in 1880.<sup>50</sup>

## 2. *Progress of Quarantine Legislation.*

While the development of local control was the distinguishing feature of this period, quarantine continued to occupy also a large share of legislative attention.

In 1811 the quarantine law was revised.<sup>51</sup> The length of time for which quarantine must be performed, previously left to the discretion of the health officer, was fixed for vessels from infected ports, or with disease on board, at 30 days; for those from tropical ports (a list was given) four days; and for those from other foreign ports (another list) two days. The law prescribed in detail the methods to be used in cleansing and disinfecting infected or suspected vessels. Otherwise the provisions of the earlier laws were unchanged.

The stringency of these measures, together with the decline in commerce caused by the embargo and the War of 1812, prevented another outbreak of yellow fever until 1819.<sup>52</sup> Probably the long immunity caused careless administration of the laws; there was another epidemic in 1819. Once the disease was recognized "the board of health of that year determined to discard the temporizing system which boards of preceding years, infected by dangerous doctrines (i. e., as to the local rather than foreign origin of the disease) and led astray by their official advisers, had too long pursued. An entire new system was put into force. It was resolved, although the disease might be introduced, to endeavor at least to stop its mortality. . . . It was looked upon as a contagious pestilence and the part of the city where it was introduced immediately and totally cleared of its inhabitants, and those who would not remove of their own accord, turned out of their houses by force."<sup>53</sup> These high-handed and unauthorized proceedings of the board of health seem to have been fairly

<sup>49</sup> P. v. Supervisors Monroe Co., 18 Barb. 567 (1854).

<sup>50</sup> *In/ra*, p. 29.

<sup>51</sup> 1811, ch. 175.

<sup>52</sup> During the war with Great Britain "the cities and towns along the whole of our sea-coast were totally exempt from the fever of the tropics."—Townsend, *op. cit.*, pp. 90, 91.

<sup>53</sup> Townsend, *op. cit.*, p. 220.



effectual in preventing a widespread epidemic; there were not more than 150 cases and 50 deaths. A new quarantine law was passed within a year,<sup>54</sup> but except for requiring reports from the health commissioners and adding a few details of quarantine there were no changes.

Three years later, upon the next appearance of the disease, a new board of health "thought themselves obliged to await the imprimatur of their official advisers before they could have recourse to precautionary measures." The resident physician "strenuously persisted it was not yellow fever" and not until the public became generally alarmed did he, "finding that the course which the board at his instigation pursued had become the subject of severe and general animadversion, at length acquiesced in the common belief nearly one month after the disease had begun, . . . stating by way of explanation that infected air, which had until then produced nothing but bilious fevers, had become sufficiently concentrated to generate yellow fever." The board finally put energetic measures into execution; they "began to depopulate and barricade that part of the city where the disease was introduced, stopping up all streets and lanes; . . . but such were the prejudices of the community against this novel procedure, first introduced in 1819, and such the fallacious hope that the disease would not spread that the board was under the necessity of adopting this measure with the greatest precaution, by which means the disease kept the start of the barricades . . . until the general panic, about September 1, caused the inhabitants to abandon their houses *en masse* and served to put a stop to the progress of the disease."<sup>55</sup>

The new health law, enacted the following March,<sup>56</sup> authorized the board of health, upon the report of a contagious disease, to quarantine the house and family; to exercise all such powers as should "in their judgment be most conducive to the public good;" and were required, "whenever a fever of a malignant, infectious, or contagious nature" should appear in any part of New York City, "to adopt, without delay, such prompt measures as will effectually prevent all communication between the part or parts so infected, and any other part of the city," even to the extent of fencing off a section of the city.

<sup>54</sup> 1820, ch. 229.

<sup>55</sup> Townsend, *op. cit.*

<sup>56</sup> 1823, ch. 71, sec. 37.

This same law drew the line of quarantine even tighter than before. Vessels bringing West Indian produce from northern ports of the United States might not escape quarantine unless it could be proved that the West Indian articles had been landed at the northern ports more than 20 days; persons who had come from the West Indies to another port in the United States between June 1 and October 1 were forbidden to enter New York City within 20 days of the date of sailing, or 15 days after the last case of sickness or death on the vessel by which they had come, nor might such persons bring in any baggage until it had been purified. This act recognizes for the first time the existence of vessels "navigated by steam" and subjects them to "such length of quarantine and such regulations as the health officer shall designate." It also extended the provisions of the act to all diseases which the board of health deemed pestilential, contagious, infectious, or otherwise dangerous to the health of the city.

At about the same time an act was passed<sup>57</sup> which, recognizing the fact that an epidemic of infectious disease had rendered communication with infected parts of the city dangerous, and that it might so happen again, permitted the service by mail of notices of protest or demand whenever the board of health should prohibit communication with those parts of the city; and the next year,<sup>58</sup> the registration of such notices was required in a book to be kept by the clerk of the city or county of New York.

The law of 1823 reached the high tide of stringent quarantine restrictions in New York, yet the friends of quarantine demanded even stricter laws: "Our health laws, which now are perhaps of too general application, . . . ought to be limited more particularly to the West Indian ports and islands, against which they should be made more rigid than at present." The same writer thought that direct intercourse with the West Indies should be entirely prohibited during the five summer months; that the existing system of quarantine was a burlesque and totally inefficient;<sup>59</sup> that "quarantine ought to be, as in other countries (e. g., Marseilles), an institution essentially military."<sup>60</sup> "All schemes, however, will have but a partial effect so

<sup>57</sup> 1823, ch. 216.

<sup>58</sup> 1826, ch. 17.

<sup>59</sup> Townsend, *op. cit.*, pp. 228, 229.

<sup>60</sup> Cf. S. C. laws, 1797, ch. 1672; 1809, ch. 1044; *infra*, Appendix A.

long as our country is unprovided with a national code of quarantine laws."

From this time on, the modifications of the quarantine laws tended gradually toward greater freedom and simplicity, toward a relaxing of existing laws rather than a stricter system. In 1830<sup>61</sup> the health officer, at his discretion, might allow "any healthy person, arriving from sea in a healthy vessel, and who had not been exposed to any infectious or contagious disease during the last 15 days preceding such arrival at quarantine, to come to the city without baggage," even if he came from the dreaded West Indies; in 1831<sup>62</sup> vessels leaving West Indian or American ports between Cape Henlopen and the equator before July 1, and which had proceeded thence to healthy ports in Europe and had had no case of yellow, pestilential, or infectious fever on board during the voyage, might be allowed to come to their wharves after such quarantine and cleansing as the board of health might designate. A further amendment in 1836<sup>63</sup> enlarged the list of places from which vessels might be permitted to approach their wharves. By 1839<sup>64</sup> the mayor, resident physician, and commissioner of health were constituted a board of appeal from the directions or regulations of a health officer, possibly too zealous, with power to grant such relief as might seem expedient or proper. Up to this time the discretion of the health officer had been practically unlimited in many matters and offered opportunity for grave abuses, particularly in regard to the licensing of lighters and granting permits.<sup>65</sup>

In 1846 petitions were presented to the legislature signed by many residents of New York City, complaining of the grievances incident to the existing quarantine laws. A committee of the House was appointed to investigate conditions and propose a new law. During the progress of this investigation the embers of the old quarrel among the doctors as to the origin of yellow fever burst out anew; both parties appeared and advanced their views, for and against quarantine. In their report the committee diplomatically found that the "quarantine regulation should be as perfect as human skill can devise with as

<sup>61</sup> 1830, ch. 333.

<sup>63</sup> 1836, ch. 230.

<sup>62</sup> 1831, ch. 304.

<sup>64</sup> 1839, ch. 114.

<sup>65</sup> "The lighterage and care of quarantined goods may now be counted among the partisan political spoils in the Empire State. And the management of that item of quarantine service has long been such as to endanger the public health and greatly embarrass and tax commerce."—*Proceedings Third Natl. Quarantine and Sanitary Convention*, 1859.

little embarrassment to commerce as is consistent with wise caution for the public health." They recommended that large discretion should be given the health officer, who should be a man of science and medical skill, and that there should be opportunity for appeal from his decisions; that the state of the vessel should be considered most important; and that healthy persons should be allowed to come at once to the city. They concluded that quarantine was invoked chiefly against yellow fever, cholera, and smallpox; that experience had proved vaccination to be the greatest preventive against the general prevalence of smallpox, more efficient than quarantine; that the value of quarantine as against cholera was doubtful.<sup>66</sup>

Following these recommendations a new law was enacted<sup>67</sup> renewing the general features of quarantine, but permitting vessels which were free from sickness, upon giving proofs that the port of departure had been free from infection at the time of sailing, to come to the wharves to discharge their cargoes after obtaining a permit from the health officer and permission from the board of health. It also gave to the health officer authority to require vaccination.

The law of 1850, while embracing provisions for the port as well as for the city of New York, was concerned chiefly with local conditions.<sup>68</sup> The only additional quarantine provisions were the grant of power to the mayor to issue a proclamation against an infected place, after which vessels from that place must undergo a quarantine of thirty days; and the grant to the board of health to regulate at their discretion internal intercourse with such places, whether by land or water.

Quarantine laws for the port of New York, therefore, during the second period made no great advances in principle or in method. The culmination of strict and detailed measures was reached in the law of 1823. Toward the close of the period there was a tendency to relax the extreme strictness of the measures which had obtained twenty years earlier.

Meanwhile quarantine legislation for the state outside of the port of New York did not advance beyond the provisions of the early laws<sup>69</sup> until 1832. New York City was considered the guardian

<sup>66</sup> *Report Com. N. Y. House on Quar. Laws*, 1846.

<sup>67</sup> 1846, ch. 300.

<sup>68</sup> 1850, ch. 275.

<sup>69</sup> 1801, ch. 86; *supra*, pp. 6, 10.



of the entire state and quarantine for the port of New York sufficient protection,<sup>70</sup> but in 1832 disease found other means of ingress. In that year cholera was brought to Quebec and Montreal by immigrants from infected European ports, and from those cities, following the path of the immigrants up the St. Lawrence River and along the Great Lakes, entered the state of New York from the north. On June 22 an emergency measure, limited to February, 1833, was passed,<sup>71</sup> giving the governor power to proclaim quarantine against places in Canada, the United States, or elsewhere, in which Asiatic cholera or other malignant disease was known to exist. It was made the duty of the common council of every city, the board of trustees of every village, in which there was not already a board of health, in counties bounded by the Great Lakes, the St. Lawrence River, Lake Champlain, or the Hudson River, or bounded or intersected by any canal, upon issuance of the governor's proclamation, to appoint forthwith a board of health and a competent physician as health officer. These local boards were to determine the length of time, the place and mode in which quarantine should be performed; to prescribe the duties of the health officer; to make regulations for the treatment and purification of persons and vessels detained, and the manner of their discharge; for the regulation of intercourse with infected places; and for the apprehension, separation, and treatment of immigrants and persons having no fixed residence in the state. They were also to make regulations for the suppression of nuisances and such other regulations as were necessary for the preservation of the public health.<sup>72</sup> They were to procure suitable places for the sick and, if necessary, supply medical aid, attendance, and provisions. And they were empowered to issue warrants for the removal of persons not otherwise subject to the regulations adopted. In other counties than those indicated the local authorities might institute boards of health if in their judgment it was expedient. The governor was empowered to appoint agents to investigate the causes of the disease in both the United States and Canada, and the best methods of prevention.

<sup>70</sup> See preamble to 1799, ch. 70; *supra*, p. 9.

<sup>71</sup> 1832, ch. 333.

<sup>72</sup> *Supra*, p. 17.



## C. THIRD PERIOD, SINCE 1855.

As the chief effort of the first period had been to exclude yellow fever, and of the second to exclude cholera, or prevent its spread, so the chief effort of the third period is toward efficiency of administration. As the means employed for the first object was quarantine and for the second the development of local boards, so the chief means for this third object is centralized supervision and control.

1. *Legislation for New York City.*

The first part of this period was coincident in New York City with the rise of Tammany Hall to its most flourishing state. As has been shown,<sup>73</sup> the law of 1850 placed almost unlimited power in the hands of the "city inspector" to carry out the regulations of the mayor and council acting as a board of health and even to execute orders of his own making. The city inspector did not long hesitate to take advantage of the political power placed in his hands by the appointment of subordinates; graft was easy, and incompetence and inefficiency well paid. The condition of matters relating to health in the city grew steadily worse. By 1859 a committee of the Senate, appointed to investigate the health department, reported that "as at present organized, the health department does not accomplish the object for which it was intended," and recommended a board of health composed in part of members of the medical profession, with a physician-superintendent of public health as chief executive officer, and a qualified practitioner as subordinate sanitary officer in each ward.<sup>74</sup> No action was taken by the legislature and things went on from bad to worse.

A vivid picture of conditions in 1865 is given in the autobiography of Andrew D. White:

The condition of things in the city of New York had become unbearable; the sway of Tammany Hall had gradually brought out elements of opposition such as before that time had not existed. . . . The city system was bad throughout; but at the very center of evil stood what was dignified by the name of the "Health Department." At the head of this was a certain Boole, who, having gained the title of "city inspector," had the virtual appointment of a whole army of so-called "health inspectors," "health officers," and the like, charged with the duty of protecting the public from the inroads of disease; and never was there a greater outrage against a city than the existence of this body of men absolutely unfit both as regarded character and education for the

<sup>73</sup> *Supra*, p. 16.

<sup>74</sup> *Report N. Y. Senate Com.*, 1859.

duties they pretended to discharge. Against this state of things there had been developed a "citizens' committee," representing the better elements of both parties—its main representatives being Judge Whiting and Mr. Dorman B. Eaton—and the evidence these gentlemen exhibited before the committee on municipal affairs, at Albany, as to the wretched condition of the city health boards was damning. Whole districts in the most crowded wards were in the worst possible sanitary condition. There was probably at that time nothing to approach it in any city in Christendom save, possibly, Naples. Great blocks of tenement houses were owned by men who kept low drinking-bars in them, each of whom, having secured from Boole the position of "health officer," steadily resisted all sanitary improvements or even inspection. Many of these tenement houses were known as "fever nests;" through many of them smallpox frequently raged, and from them it was constantly communicated to other parts of the city. Therefore it was that one morning Mr. Lainbeer, the only Republican member from the city, rose, made an impassioned speech on this condition of things, moved a committee to examine and report, and named as its members Judge Munger, myself, and the Democratic senator from the Buffalo district, Mr. Humphrey.

The examination was long and complicated, and "brought to view a state of things even worse than anything any of us had suspected." As the other two members of the committee were not re-elected there was no report,

but the committee on municipal affairs having brought in a bill to legislate out of office the city inspector and all his associates and to put in a new and thoroughly qualified health board, I made a carefully prepared speech, which took the character of a report. The facts brought out were enough to condemn the whole existing system twenty times over. By testimony taken under oath, the monstrosities of the existing system were fully revealed, as well as the wretched character of the "health officers," "inspectors" and the like . . . their appointments being made not to preserve the public health but to carry the ward caucuses and elections.

As a result of this exposure, an act was passed<sup>75</sup> for the creation of the "Metropolitan Sanitary District," embracing the same territory as the "Metropolitan Police District" already established. In control of health matters within the district was a "Metropolitan Board of Health." This board was composed of nine persons: four "sanitary commissioners" appointed by the governor and Senate, three of whom must be physicians, and one a resident of Brooklyn; the health officer of the port; and the four Metropolitan Police Commissioners. The board was given power to create a chief executive office, entitled the "Sanitary Superintendent's Office," and to appoint an experienced physician to fill it; also two assistant superintendents; and sanitary inspectors, not to exceed 15, 10 of whom must be physicians; also clerks and servants as necessary. From time to

<sup>75</sup> 1866, ch. 74.

time they might engage the services of a sanitary engineer. Reports in writing were required weekly from the superintendents and inspectors. All authority, duty, and power for the purpose of preserving or protecting life or health or preventing disease exercised by the former boards of health, commissioners of health, or city inspector of New York, Brooklyn, or anywhere within the district, were now conferred upon the new board; and the existing boards, city inspectors' office and all adjuncts thereto abolished. Besides transferring all existing powers to the new board, the legislature vested in it wide quasi-judicial and summary powers for the abatement of nuisances; thus it might issue warrants, compel witnesses, administer oaths, and determine a question upon a hearing after notice to the interested party; it might enforce its own orders over sewerage, drainage, ventilation, noxious trades, over premises, buildings, vehicles, vessels, and infected articles and places; it might enact, alter, amend, or annul by-laws, rules, and regulations for the protection of health and the subjects mentioned above, but all such ordinances must be published before being put into effect. In the "presence of great and imminent peril to the public health . . . by reason of impending pestilence," it was made the duty of the board to "take such measures and to do and order and cause to be done such acts and make such expenditures for the preservation of the public health, as it may in good faith declare the public safety and health to demand, and the Governor of the state shall in writing approve," but the exercise of this extraordinary power for expenditure required, besides the governor's assent, the written assent of six members (two-thirds) of the board. The law required mutual information and co-operation between the board of health and the quarantine officials for the prevention of the spread of disease and the protection of life and health, and between the board of health and the police board for the enforcement of sanitary rules, regulations, and orders; and mutual information and useful suggestions between the Metropolitan Board and local boards throughout the state. As far as possible without serious expense, the board was required to gather such information relating to disease and health as might be useful in the discharge of its duties and contribute to the promotion of health in the state. Annual reports containing the information so gathered, together

with vital statistics, and an account of the actions taken during the year, were to be presented to the governor along with suggestions for further legislation. The board was authorized to supply facilities for gratuitous vaccination and disinfection; to afford medical relief among the poor; to remove and isolate cases of contagious disease; also to require reports and information from public dispensaries, hospitals, prisons, schools, and all other public institutions; and when needed also from managers of theaters and other places of resort and amusement. An amendment within two months<sup>76</sup> interpreted the powers of the board to include the ordering and enforcing of repairs on buildings; the regulation and control of public markets as to cleanliness, drainage, ventilation, and sale of improper articles; the removal of obstructions on streets; the regulation and licensing of scavengers; the prevention of accidents; and generally the abatement of all other nuisances. In 1867 further amendments<sup>77</sup> gave the board power to regulate the time and manner of driving cattle through the streets; defined more fully the procedure to be followed in suits arising under the act; and, on account of decisions of the courts denying the right of the board to declare that a nuisance which was not such at common law, defined a nuisance as embracing not only "public nuisance" as known in common law or equity jurisprudence, but also "whatever is dangerous to human life or detrimental to health," buildings overcrowded with occupants, unprovided with adequate exits, insufficiently supported, ventilated, sewerred, drained, cleaned, or lighted; "and whatever renders the air or human food or drink unwholesome."

Almost immediately after the organization of the new board in the summer of 1866 the cholera again made its appearance, and for the last time gained a large foothold in the country. The board of health exercised such vigorous measures, however, that the epidemic was less serious in New York than on former occasions and there were only a few cases. These same vigorous measures, together with the animosity of Tammany, soon brought an attack upon the constitutionality of the act.<sup>78</sup> Indeed in the four stormy years of its existence, the Metropolitan Board was rarely out of court. During that time, however, the sanitary condition of the city was transformed, and

<sup>76</sup> 1866, ch. 686.

<sup>77</sup> 1867, chs. 700, 956.

<sup>78</sup> *Infra*, p. 95.



never since has the lower element among the politicians dared to debase the health officers to the same extent as previously.

In 1870 the legislature passed an act for the reorganization of the government of New York City.<sup>79</sup> This act created a "health department" consisting of the four police commissioners, the health officer of the port, and four health commissioners to be appointed by the mayor for five years, two of whom must be practicing physicians. In this department there were to be four bureaus: the office of city sanitary inspector, of which the incumbent must have been a practicing physician for 10 years; the sanitary permit bureau; the street cleaning bureau; and the bureau of records. To the department thus created were transferred all powers and duties of the Metropolitan Board within the city with power to adopt and alter the ordinances of that board as the sanitary code of New York City.<sup>80</sup>

The new charter of New York City in 1873<sup>81</sup> reduced the number of members of the board to four: the president of the board of police; the health officer of the port; and two commissioners of health, one of whom must have been a practicing physician for five years. The number of bureaus was reduced to two: the head of the first was to be the sanitary superintendent, who must have been a practicing physician for 10 years and three years a resident of the city, and who was to be the chief executive officer of the department; the head of the other bureau was the register of records. The board as so constituted succeeded to the powers and duties of the last board and in addition might appoint an attorney.

The New York City consolidation act in 1882 did not add anything to the existing health laws, but codified them after the manner of revised statutes.<sup>82</sup>

Since that time amendments have increased the number of inspectors<sup>83</sup> and have increased the powers of the department with regard to drainage, plumbing, water supply, cleanliness, ventilation, overcrowding, and ordering the vacation of buildings unfit, in their judgment, for human habitation.<sup>84</sup>

The charter of Greater New York in 1897 made no changes in

<sup>79</sup> 1870, ch. 137, Art. XI.

<sup>80</sup> 1870, ch. 383.

<sup>81</sup> 1873, ch. 335.

<sup>82</sup> 1882, ch. 410; ch. 2, sec. 41; ch. 3, secs. 47-52; ch. 12, secs. 533-667; ch. 25, secs. 2026-31.

<sup>83</sup> 1885, 1887.

<sup>84</sup> 1887, ch. 84; 1888, ch. 422; 1889, ch. 211; 1890, ch. 486.



the existing law other than those necessary to adapt it to the new conditions; nor have there been any essential changes since that time. The law of 1866 as amended in 1866 and 1867 has furnished the substantial basis for the duties and powers of board and department ever since that time; it explained these powers and duties in great detail and gave ample means for their enforcement; if the city of New York is not in proper sanitary condition, the fault lies not with the law but in the lack of efficiency and public spirit of those called upon to administer the law.

## 2. *The State Board of Health.*

As has been already seen, the cholera act of 1832 gave the governor power to "employ suitable agents to proceed to any part of this state, or to Upper or Lower Canada, for the purpose of procuring information in relation to the progress of the said disease, and the prevention or treatment thereof, or for any other purpose he may deem conducive to the public health." Here is found the germ of the idea—agents of the state to investigate diseases, especially epidemics, as to their causes and the best methods of prevention—which later developed into state boards of health.

After the middle of the nineteenth century the germ theory of disease began to be accepted and the need for scientific information and advice with regard to matters affecting the public health began to be felt. In 1849, during an epidemic of cholera, Massachusetts appointed a commission for the sanitary survey of the state, but did not follow their recommendation, to establish a state board of health, for 20 years, until 1869.<sup>85</sup> After Massachusetts had led the way other states were not long in following; within six years seven other states had created state boards. In 1872 a number of men keenly interested in the public welfare, mainly physicians, formed in New York City the "Public Health Association" for the presentation and discussion of carefully prepared papers upon subjects connected with the public health. The necessity of state boards of health was urged by Dr. Elisha Harris of New York in a paper before the association in 1873<sup>86</sup> and the draft of a law was prepared and

<sup>85</sup> *Infra*, p. 57.

<sup>86</sup> "A central source of information and of advisory as well as ultimate authority should be created and put into operation in connection with local authorities."—*Public Health*, 1, p. 472.

recommended by the association to the legislatures of the various states. But the states are notoriously slow to act in health matters unless spurred on by impending pestilence, and it was seven years before the legislature of New York acted upon the recommendation of the association.

In 1880<sup>87</sup> the state board of health was established by an act which followed closely the draft prepared by the Public Health Association. The board was to consist of nine persons: three of these, called the commissioners of health, of whom two must be physicians of seven years' standing, were to be appointed by the governor; three, the attorney-general, the superintendent of the state survey, and the health officer of the port of New York, were *ex-officio* members; and three were to be chosen by the governor from among the commissioners or members of boards of health of cities of the state, one from New York City. The expenses of the board were to be paid but no compensation allowed except for the secretary, who was to be chosen by the board either from among their number or otherwise. The duties of the board were threefold: to make investigations and collect and preserve information as to disease, especially epidemics, and publish the results obtained; to collect and preserve vital statistics; and, upon request of the governor, "to examine into nuisances and questions affecting the security of life and health in any locality;" upon an unfavorable report by the board the governor was empowered to order such nuisances abated or removed. By a later amendment<sup>88</sup> such an order was made presumptive evidence of the existence of the nuisance and a penalty was attached for maintaining it after notice of the order for abatement.

In 1883<sup>89</sup> the duties of the board were increased to include, upon complaint of three residents, the examination of cases of disease said to be caused by overflow and leakage of canals. Upon the report of the board that such a nuisance was dangerous to health, the superintendent of public works was empowered to abate it.

The first actual power attained by the board was in 1885<sup>90</sup> when it was given power to make regulations protecting from contamination all public supplies of potable waters and their sources within the

<sup>87</sup> 1880, ch. 322.

<sup>88</sup> 1882, ch. 308.

<sup>89</sup> 1883, ch. 291.

<sup>90</sup> 1885, ch. 543; 1888, ch. 52; 1892, ch. 235.

state; but even then such regulations were to be in force only after approval by the county judge of the county in which the waters were situated; and the methods of enforcement were clumsy, no direct power being given to the state board beyond ordering the local boards to enforce obedience.

In the same year we find the beginning of supervision by the state board over the local boards in the provisions of an act<sup>91</sup> primarily for local boards, in which the state board was given power to request special meetings of local boards; to take control of the local registration of vital statistics whenever it was not being properly made; and to compel by mandamus the establishment of local boards and the appointment of local officers upon failure of the local authorities to act, and the performance of any duty required of local officers by the law. In 1888 the board was given the same power to issue subpoenas and compel witnesses as judges of the Supreme Court.<sup>92</sup>

By the Public Health Law in 1893,<sup>93</sup> in case any municipal corporation omitted to establish a board of health, the state board was further empowered to exercise the powers of a local board in that locality and to appoint a health officer and fix his duties and compensation until such time as the regular authorities should act.

From being a purely investigative body, the state board of health had gradually acquired executive and quasi-legislative functions until it had become an important and powerful body. One more power, a quasi-judicial one, was added in 1895:<sup>94</sup> that of administering oaths and compelling witnesses. But the board was unwieldy, the duties were performed chiefly by the secretary, and in 1901 it was abolished and the state department of health created to fulfil the same functions.<sup>95</sup> At the head of the state department of health there is a salaried commissioner of health to whom are intrusted all of the powers and duties formerly exercised by the state board of health.

Since its establishment, the powers of the department have been further increased in various ways, not all bearing directly upon health matters. It has received power to examine into the inforce-

<sup>91</sup> 1885, ch. 270.

<sup>92</sup> 1888, ch. 146.

<sup>93</sup> 1893, ch. 661; cf. Pa. 1885, ch. 37; S. C. 1878, ch. 610. See Appendix A.

<sup>94</sup> 1895, ch. 928.

<sup>95</sup> 1901, ch. 29.

ment of the tenement-house laws in cities of the first class;<sup>96</sup> to control the discharge of sewage or waste from mills and industrial establishments into the waters of the state;<sup>97</sup> and, a most important step toward centralization, to appoint the local health officers of all municipalities except cities, on the nomination of the local boards of health.<sup>98</sup>

### 3. *Local Boards of Health.*

At the end of the last period, as we have seen, the law of 1850 made it the duty of all municipal authorities to appoint a board of health and a health officer. Few villages, however, and not all of the cities complied with the act, and there was no method of compelling obedience. There was also a general lack of legislative interest until 1867, when, after another visitation of cholera in 1866, an amendment to the law of 1850 increased the powers of local boards over nuisances.<sup>99</sup>

It had been held by the Supreme Court of New York in 1854 that boards of health under the act of 1850 could not sue or be sued<sup>100</sup> and that orders for the removal of nuisances could not be enforced by the infliction of penalties.<sup>101</sup> The first defect was remedied by the amendment of 1867, by which power was given to make orders in special cases, and to enforce them by summary abatement. A further amendment in 1870<sup>102</sup> permitted local boards of health to impose penalties for violations of or non-compliance with their orders or regulations, to maintain actions in court to collect such penalties, or to restrain by application for injunction.

In 1881, after the creation of the state board of health, the law was again amended. Relations with the state board were established by requiring reports to the state board of facts regarding infectious and epidemic diseases, and by requiring meetings of town boards at the request of the state board. In case of negligence on the part of local authorities in appointing boards of health or in filling vacancies it became the duty of the county judge to fill such vacancies, but, this clause has since been declared unconstitutional.<sup>103</sup> All local

<sup>96</sup> 1901, ch. 283.

<sup>98</sup> 1903, ch. 383.

<sup>97</sup> 1903, ch. 468.

<sup>99</sup> 1867, ch. 790.

<sup>100</sup> P. v. Supervisors Monroe Co., 18 Barb. 567 (1854).

<sup>101</sup> Reed v. P., 1 Park Cr. 481.

<sup>102</sup> 1870, ch. 559.

<sup>103</sup> P. v. Houghton, 182 N. Y. 301 (1905).



boards were given increased powers for quarantine and isolation, and it was made their duty to provide vaccination for all those needing it; to supervise and complete the registration of vital statistics; and to make regulations for burial permits.<sup>104</sup>

In 1885<sup>105</sup> a new law for local health authorities was passed, repealing previous laws. The substance of the laws as to structure, powers, and duties of boards of health were not changed—the changes concerned chiefly the relations between the local and state boards. Notice of the membership and organization of local boards must be sent to the state board; special meetings of city and village boards as well as of town boards might be requested by the state board; the quality and source of vaccine virus supplied by local boards must be approved by the state board; registration of vital statistics must be upon forms prescribed; and the state board might by mandamus compel the establishment of local boards and the performance of their duties under the act. It was only five years since the establishment of the state board for purposes of investigation only, yet the ideal of “ultimate authority” presented by Dr. Harris in 1873 was fast being realized.

From the passage of this act until the codification of the health laws by the Public Health Law in 1893, the only changes of consequence were in 1888<sup>106</sup> when local boards were given power to issue subpoenas and compel witnesses like justices of the peace; and in 1892<sup>107</sup> when all health departments and commissioners were given power to enforce an act to prevent the rendering of fat as a nuisance.

The Public Health Law of 1893<sup>108</sup> codified all the existing health laws but did not modify the laws governing localities in any essentials. Since that time various minor modifications have been made in the structure of the boards in number of members, length of term, etc.; as to health officers, who from 1895–97 were appointed in cities by the mayor and council but, before and after those dates, by the board of health;<sup>109</sup> but no essential changes were made until 1903,<sup>110</sup> since which time the appointment of health officers for all municipalities except cities has been by the state commissioner upon nomination

<sup>104</sup> 1881, ch. 431.

<sup>105</sup> 1885, ch. 270.

<sup>106</sup> 1888, ch. 146.

<sup>107</sup> 1892, ch. 646.

<sup>108</sup> 1893, ch. 661.

<sup>109</sup> 1895, ch. 584; 1897, ch. 282.

<sup>110</sup> 1903, ch. 383.



of the local board. The powers and duties of village boards have been increased by giving them power to recommend additions and alterations to the sewage system, which it became the duty of the trustees to carry out only after approval by both the trustees and the state board of health;<sup>111</sup> to condemn and destroy infected articles made in tenement houses where disease existed;<sup>112</sup> and to require hermetically sealed coffins for the transportation of the bodies of those who had died of contagious disease.<sup>113</sup>

It will be seen that the changes made in the powers and duties of local boards since 1850 are neither many, nor, with few exceptions, important; the important fact is found in their increasing dependence upon the state authorities.

#### 4. *Quarantine since 1850.*

During the period from 1850 to the present time there have been only four laws of any importance bearing upon the subject of quarantine. The first of these in 1856<sup>114</sup> differed from that of 1850 only in the simplification of the classes of vessels subject to quarantine; in forbidding the detention of vessels free from infection beyond the time necessary for examination, and in giving to the health officer authority to vaccinate all persons under quarantine whenever, in his judgment, it was necessary for the preservation of the public health.

Yellow fever had been brought in vessels from infected ports during nearly every summer, but, since 1822, had been kept from gaining any foothold until 1856. During that summer, owing to bad management, there were over five hundred cases, all within five miles of the quarantine station on Staten Island. This led to a frantic demand by the inhabitants of the island for the removal of the station to some other place.<sup>115</sup> An act was passed in 1857<sup>116</sup> providing for the removal and for securing a new site, but there was difficulty in finding a site where the same objections could not be raised, and when, in 1858, nothing more had been done about removing the station, the Staten Islanders took matters into their own hands, invaded the buildings of the hospital, carried out the sick, and burned

<sup>111</sup> 1895, ch. 928.

<sup>112</sup> 1899, ch. 191.

<sup>113</sup> Richmond, *New York and Its Institutions*, p. 190, 1871.

<sup>114</sup> 1899, ch. 211.

<sup>115</sup> 1856, ch. 147.

<sup>116</sup> 1857, ch. 68.

all the buildings. The quarantine officials made use of hulks of old vessels until the legislature should take further action.

In 1863<sup>117</sup> the legislature passed a law putting the whole quarantine establishment upon a new basis. It was to consist of a boarding-station, an anchorage for vessels, a floating hospital, docks and wharves, and residences for officers and men. Three commissioners of quarantine, appointed by the governor for three years, were to be the custodians of the establishment, with general oversight of the erection of the new buildings, docks, wharves, and floating hospital. Certain small islands in the outer harbor were enlarged for the accommodation of the necessary buildings. By amendment in 1865 the commissioners were given also power to make rules and regulations for the protection of the quarantine establishment and for the government of the employees. A health officer, a physician of 10 years' practice appointed by the governor, was to execute the law, with power to appoint or dismiss at pleasure two assistants and such other employees of the floating hospital and boarding-station as might be found necessary, and, together with the commissioners of quarantine, to license lightermen, stevedores, and others. Quarantine of vessels was limited to those from an infected port, or with an actual case of quarantinable disease on board or during the voyage, although vessels from southern or suspected ports were subject to examination. For the first time a list of quarantinable diseases was embodied in the law, and consisted only of yellow fever, cholera, typhus or ship fever, smallpox, and any "new disease of a pestilential nature" at the discretion of the health officer and commissioners. No well persons might be detained longer than necessary to secure cleanliness, unless recently exposed to smallpox, when they might be vaccinated and detained "until the vaccine should have taken effect." Merchandise from infected ships was divided into three classes: the first subject to an obligatory quarantine and purification; the second, to quarantine at the option of the health officer; and the third, exempt from quarantine. In addition to executing these provisions of the law, it was made the duty of the health officer "in the presence of immediate danger to take the responsibility of applying such additional measures as may be deemed indispensable for the protection of the public

<sup>117</sup> 1863, ch. 358.

health." He was also given power to detain and require the purification of vessels in an unhealthy state, although without an actual case of quarantinable disease on board. He might call upon the metropolitan police to aid him in any emergencies. Later, in 1865,<sup>118</sup> police were appointed to serve directly under the health officer.

This law of 1863 continued until 1892 with the amendment of certain details in 1865, 1866, and 1867, and became the model for quarantine laws in other maritime states, notably Louisiana.

The next important act was the supplementary act of 1885,<sup>119</sup> passed at the recommendation of the state board of health,<sup>120</sup> which made it the duty of the health officer to require bills of health from masters of all vessels from a foreign port, countersigned by the United States consul at that port, and setting forth in detail the sanitary condition of both the vessel and the port of departure. This act increased the list of quarantinable diseases by the addition of "measles, scarlatina, diphtheria, relapsing fever, and any disease of a contagious, infectious, or pestilential character which shall be considered by the health officer dangerous to the public health;" and also permitted the examination of all persons as to their protection from smallpox and their vaccination or detention.

In 1892, in view of the probable approach of cholera from Europe, a new quarantine law was passed<sup>121</sup> which changed the form rather than the substance of the law of 1863 and was evidently drawn with haste and carelessness. The quarantine establishment was increased by a stationary hospital and such other places and structures as might have been authorized by law. The salary of the health officer was fixed at a definite amount for the first time and no longer depended upon the fees received. The section of the law of 1863 which conferred extraordinary powers in time of danger was copied verbatim and became shortly the subject of great discussion.

In early September several steamers arrived from Hamburg, where cholera was raging, with cases on board. The floating hospital and those on Swinburne and Hoffman Islands were not sufficient to hold all the passengers detained. The health officer, overriding the protests of the local board of health, landed the sick and those

<sup>118</sup> 1865, ch. 592.

<sup>119</sup> 1885, ch. 534.

<sup>120</sup> *Report State Bd. H.*, 5, p. 23.

<sup>121</sup> 1892, ch. 486.

detained on account of exposure on Fire Island in the town of Islip.<sup>122</sup> Immediately a double storm burst about his head, on one side from those detained, on account of insufficient accommodations, exposure, and personal loss; on the other from the inhabitants of the town in which the suspected passengers had been landed. Suits were brought, but the action of the officer was sustained in the case of *Young v. Flower*<sup>123</sup> and the others were dropped. In the codification of the health laws in 1893 the section conferring extraordinary powers "in the presence of immediate danger" has been reaffirmed with the addition of the words: "of which he shall be the judge."

Since 1892 there has been no important change in the quarantine law. In 1900 the floating hospital was abolished and vessels from all domestic ports were made subject to examination and detention. Also the provision allowing vessels subject to quarantine to put again to sea without breaking bulk was withdrawn.

Reviewing the quarantine legislation of the past sixty years, we find many changes in the quarantine establishment. The present laws go less into detail as to the list of places, seasons of quarantine, and methods of purification. Healthy persons and healthy vessels are no longer made to undergo an arbitrary quarantine, the unnecessarily stringent requirements of 1823 have been abolished, the broad principle of exclusion of infected persons and things alone remains. The quarantine of the port has been since 1863 under the management of officials entirely separate from those who control the local quarantine of the city and the isolation and removal of cases of infectious disease.

##### 5. *Legislation with Regard to Vaccination.*

There is another phase of legislation to be considered in the fight of a state against disease: that which is concerned with the theories of preventive medicine or serum therapy. These theories began to be noticed by the legislature in the latter part of the eighteenth century and are likely to prove the instigating cause of the chief laws of the future. The first instance of the practice of these theories had been the inoculation for smallpox, which had been extensively practiced in Massachusetts.<sup>124</sup> In New York the practice was for-

<sup>122</sup> *Review of Reviews*, 6, pp. 262, 343, 393, 654, 729.

<sup>123</sup> 27 N. Y. Supp. 332 (1893).

<sup>124</sup> *Infra*, p. 47.



bidden within half a mile of any dwelling-house in Cortlandt Manor, Westchester County, from 1763 to 1785;<sup>125</sup> and entirely forbidden at any place in the state for a few months during 1778.<sup>126</sup> The modern practice of vaccination, as originated by Jenner, was first introduced into New York by Dr. Valentine Seaman early in the nineteenth century;<sup>127</sup> but there was no legislative recognition of the new theory until 1844 when a "Vaccine Institution" was incorporated.<sup>128</sup> In 1846 the health officer was allowed to vaccinate any person on a vessel at quarantine whenever he thought it necessary,<sup>129</sup> and a similar power has been given in all quarantine laws since that time. In 1860<sup>130</sup> school boards and trustees were directed to exclude from the common schools any child who had not been vaccinated, with power to provide free vaccine virus for children whose parents were not able to procure it. They might appoint a physician who should ascertain the facts as to the vaccination of children of school age, provide the pure virus, and give certificates which should be evidence of compliance with the law. The provisions of this law were continued in the Public Health Law in 1893.<sup>131</sup>

In 1895 the constitutionality of the law was attacked in the case of Walters<sup>132</sup> but was upheld upon the ground that a common-school education was a privilege and not a right, and that, since this privilege was created by legislative enactment, the state could require vaccination as a precaution essential for the preservation of the public health.

There has been no change in the law regarding vaccination since that time. New York has never taken so advanced a stand as to require the vaccination of all persons within the state.<sup>133</sup>

It may not be inappropriate to forecast the future of health legislation in New York as indicated by the present tendencies. Up to 1880, when the state board of health was established, there were no pathological investigations under state auspices. As has been seen, the primary function of the state board of health was to investigate the causes of disease, and, although other functions have been added,

<sup>125</sup> 1763, ch. 1213; 1767, ch. 1332; 1775, ch. 1725.

<sup>126</sup> 1778, ch. 36; cf. Mass., *infra*, p. 47.

<sup>127</sup> *Mem. Hist. N. Y.*, 4, p. 388.

<sup>128</sup> 1844, ch. 115.

<sup>129</sup> 1846, ch. 300, sec. 16.

<sup>130</sup> 1860, ch. 438.

<sup>131</sup> 1893, ch. 661.

<sup>132</sup> 84 Hun 457.

<sup>133</sup> Cf. Mass., *infra*, pp. 50, 82.



this remains an important object of the department. To the purely investigative work of the department have been added the manufacture and distribution of various antitoxins. In this connection, it is interesting to note progress as indicated by the appropriations made. In 1880, upon the establishment of the state board, the expenses of the board were limited to \$15,000 for all purposes; in 1907,<sup>134</sup> aside from salaries, equipment, and traveling expenses, \$18,000 was appropriated for the Cancer Laboratory, \$18,000 for the manufacture of antitoxins and investigations of other serums, \$10,000, for the establishment of a hygienic laboratory, and the use of the Bender Laboratory, \$7,500 for the suppression of epidemics, and \$1,000 for a traveling tuberculosis exhibit and the sanitary instruction of health officers—a total of \$54,500. The stress laid upon the manufacture and use of antitoxins leads clearly to the opinion that the legislation of the future, besides endeavoring to exclude diseases by means of quarantine, and to limit their spread by increased efficiency of administration in regard to sanitary conditions, will furnish means to fortify the individual to resist attacks of disease as far as the progress of medical science allows. Pointing toward this has been the legislation with regard to vaccination; the tendency of both medicine and legislation is toward preventive as well as curative measures. Possibly the time will come when the legislature, going beyond the roll of honor established by law in Louisiana for the board of health which should exclude infectious diseases,<sup>135</sup> will establish punishments for a board of health of a city which permits disease to become epidemic within its limits. Indeed, already in Massachusetts penalties are prescribed for a city which does not erect an isolation hospital at the request of the state board.<sup>136</sup>

<sup>134</sup> 1907, chs. 577, 578.

<sup>135</sup> La. 1821, ch. 92; *infra*, p. 69, note 52.

<sup>136</sup> Mass. 1901, ch. 171; *infra*, p. 59.

## CHAPTER 2.

### LEGISLATION IN MASSACHUSETTS.

LEGISLATION in Massachusetts does not, as in New York, fall into distinct periods. Local government was so soon established and bore so important a part in the life of the colony that it absorbed the functions commonly performed in other colonies and states by the central government. This was true in regard to health as in other matters.

From the beginning of the Plymouth settlement the colonists had suffered as much from sickness as from the hardships incident to a new country. Even before the arrival of the colonists at Plymouth the Indians of that neighborhood had been nearly exterminated by a frightful epidemic, possibly smallpox, in 1618. There was such an epidemic of smallpox in 1631 which destroyed entire villages. In 1639 and again in 1654 a general fast was ordered on account of smallpox and fevers. But smallpox, although one of the most frequent causes of epidemics in Massachusetts, was not the only one. The long voyage across the Atlantic in badly equipped and poorly provisioned ships often induced ship fever or typhus, and tropical neighbors in the West India Islands soon sent the scourge of yellow fever. Against such enemies the weapon of quarantine was used at a very early day—less than 30 years after the first permanent settlement was formed.

#### A. QUARANTINE LEGISLATION.

In Massachusetts, as in New York, the first quarantine was performed upon orders in council. John Winthrop, in his contemporary history of those early days,<sup>1</sup> tells that in 1647 there was "a great mortality, that in Barbadoes there died six thousand, and in Christopher, of English and French, near as many, and in other islands proportionable. The report of this coming to us by a vessel which came from Fayal, the court published an order. . . . "

<sup>1</sup> Winthrop, *Hist. New Eng.*, 2, p. 313.

This order was probably the first quarantine regulation on the North American continent, and as such deserves to be given in full:

For as much as this Court is credibly informed yt ye plague, or like greivous infectious disease, hath lately exceedgly raged in ye Barbadoes, Christophers, and other islands in ye West Indies, to ye great depopulatg of those, it is therefore ordred, yt all our own or othr vessels come from any pts of ye West Indies to Boston harbor shall stop and come to an anchor before they come at ye Castle, undr ye poenalty of 100*£*, and that no pson comeing in any vessell from the West Indies shall go a shore in any towne, village, or farme, or come within foure rods of any othr prson, but such as belongs to the vessels company yt hee or shee came in, or any wayes land or convey and goods brought in any such vessels to any towne, village, or farme aforesaid, or any othr place wth'in this jurisdiction, except it be upon some island where no inhabitant resides, without licence from ye councill, or some three of them, undr ye aforesaid poenalty of a hundred pound for evry offence.<sup>2</sup>

Nearly two years later we find "The Court doth think meete that the order concerning the stoping of West India ships at the Castle should hereby be repealed, seeing it hath pleased God to stay the sickness there."<sup>3</sup> In spite of the precautions observed, however, malignant fever, probably yellow fever, was epidemic in 1647 and again in 1665.<sup>4</sup>

A similar quarantine order, called a warrant, was issued in October, 1665,<sup>5</sup> on account of the great plague in London, forbidding vessels from England to pass the castle until permitted to do so by the governor and major-general. The same temper of mind was shown which a hundred years later cropped out in the order to "trust in God and keep your powder dry;" in this case the quarantine was ordered "that so wee may be found in all due ways subservient to Providence for the preventing infection by the pestilence" and was followed by the ordering of a day of humiliation and prayer.

Selectmen of towns, too, under the very general authority conferred upon them sometimes made orders regarding sickness, as when the selectmen of Salem in 1678 ordered a certain person sick of smallpox not to come abroad for three weeks.<sup>6</sup>

Following the devastation by the "Barbadoes distemper" in Philadelphia in 1699, the colony of Massachusetts, as well as that of Pennsylvania, passed a quarantine law early in 1700. Owing probably to the presence of the proprietary, William Penn, in his

<sup>2</sup> *Records of the Colony of Mass. Bay in New Eng.*, 2, p. 237.

<sup>3</sup> *Ibid.*, 2, p. 280.

<sup>4</sup> *Report Mass. Sanit. Commis.*; Webster, *Hist. Epid. Dis.*

<sup>5</sup> *Records of Colony of Mass. Bay*, 4, p. 280.

<sup>6</sup> *Report Mass. Sanit. Commis.*, p. 48.

province at the time and to his strong personal influence with the home government, the Pennsylvania law did not meet the fate of the Massachusetts attempt, which was disallowed in Privy Council at the request of the Lords of Trade. The attempted law was quite similar to the quarantine order of 1647. It forbade ships from infected ports to come above the castle of any port of the colony without a license from the governor or two justices, nor to suffer anyone to land nor to board the vessel under penalty of £100. The reasons given for the disallowance were:

There is no such act as this (that we know of) in any other of his Majesty's plantations; And by the uncertain interpretation that may be put upon the terms Contagious, Epdemicall, and Prevailing Sickness, we think it may be liable to great abuses; The penalties thereby inflicted seem to us too high. And we are therfore humbly of opinion that the inconvenience thereby intended to be prevented may be better provided against by order of the Governor and Council from time to time than by any standing Act of the General Assembly.

Two years later, however, the subject was again taken up and the law then passed became the basis of all further quarantine legislation in Massachusetts.<sup>7</sup> This law really consisted of two parts, each of which was followed by a series of laws: one part was concerned with land quarantine, removal, and isolation; the other with maritime quarantine. By the first part selectmen of towns were empowered to preserve the health of the inhabitants by removing and isolating any persons, whether inhabitants of the town or coming from abroad, who were sick with or "late before have been visited" with plague, smallpox, pestilential or malignant fever, "or any other contagious sickness, the infection of which may probably be communicated to others." "Housing, nurses, tendance and other necessities" might be impressed upon warrant of a justice of the peace. Expenses were to be paid by the patient if possible, otherwise by the town to which he belonged.

In the other part of the law, concerned with maritime quarantine, it was enacted that upon notice or information to any justice of the peace of the arrival in a port or harbor of his county of a ship with persons, whether seamen or passengers, visited with plague, smallpox, pestilential or malignant fever during the voyage, or from a port where such sickness was common he should "forthwith take care to prevent

<sup>7</sup> 1701-2, ch. 9.

and restrain" all such persons from coming on shore, or if already on shore to send them on board again, and to restrain others from going on board; and to that end he might make out a warrant to the sheriff or constable. Information of such action was to be sent to the governor, who, with the advice and consent of the council, was to make such further orders therein as he thought fit for preventing the spread of infection.

Following first along the line of quarantine legislation with regard to vessels bringing disease, we find that in June, 1716, a committee of the council<sup>8</sup> was appointed to investigate a suitable place for a hospital for contagious diseases, and that in August, 1717,<sup>9</sup> a part of Spectacle Island was bought for that purpose and a "province hospital" erected.

A law in 1717<sup>10</sup> referred to the fact that such a province hospital had been erected and directed the keeper of the lighthouse and the commanding officer of Castle William to order masters of vessels with infectious sickness to anchor near the hospital for the removal of the sick and of infected articles. No vessel was to leave the anchorage, nor any person, nor any part of the cargo to be put on shore without leave obtained from the governor and council or from two justices of the peace and the selectmen of Boston. If any vessel with infectious sickness arrived at any other port of the province the justices and selectmen were empowered to order it to the province hospital unless such order would cause great damage, in which case they were to take measures according to the act of 1701-2. No penalty was attached to this last clause until 1757.<sup>11</sup>

With minor changes in the wording necessitated by the transfer of the province hospital from Spectacle to Rainsford Island, with the addition to the powers of the selectmen of compelling answers to questions under oath, and of requiring a written permit before vessels which had been detained could leave quarantine, this law was renewed from time to time, and became the basis of the permanent law of 1797. That it was not fully adequate to meet all the exigencies which might arise was shown by the passage of an act in 1721-22,<sup>12</sup> limited to three years, which, after stating that the plague was preva-

<sup>8</sup> *Council Records*, 10, pp. 122, 123.

<sup>9</sup> *Council Records*, 10, pp. 141, 142; *Acts and Resolves of the Province of Mass. Bay*, 2, note, p. 95.

<sup>10</sup> 1717-18, ch. 14.

<sup>11</sup> 1757, ch. 13.

<sup>12</sup> 1721-22, ch. 3.



lent in France and the ports of the Mediterranean, ordered a quarantine of 40 days for all vessels from those ports, forbade them to unlade their cargoes, and made a refusal of the captain to observe the quarantine a capital offense. The inadequacy of the existing laws was further shown by the passage of a temporary resolution on account of the epidemic of yellow fever in Philadelphia in 1793.<sup>13</sup> This resolution requested the governor to issue a proclamation requiring sheriffs, selectmen, and others to take effectual measures to prevent the introduction and spread of the disease and "whereas . . . the existing laws may not be fully competent to justify the measures necessary to be adopted on the present alarming occasion," it authorized selectmen to appoint a health officer and gave them power to detain and examine persons and goods suspected of infection.

The other portion of the act of 1701-2, giving power to selectmen of towns to remove, isolate, and provide for persons sick or infected with contagious disease, intended particularly to prevent the spread of smallpox, served its purposes only moderately well. Smallpox was almost always present and had reached epidemic proportions four or more times before that act had been passed, i. e., 1639, 1677, 1678, 1702. On one of these occasions, 1678, the number of deaths in the state reached nearly 800. At a later date, 1721, more than half of the people of Boston were sick with smallpox. In a still later year, 1792, with less than 20,000 population of Boston, 10,655 had previously had the disease, 262 left the city, and of those remaining who had not had it only 221 escaped! It is no wonder that the population of the city remained stationary during the greater part of the eighteenth century!<sup>14</sup>

The fact that smallpox was epidemic in New York in 1739 and

<sup>13</sup> Resolve 51, 21 September 1793.

<sup>14</sup> The population of Boston in 1710 was nearly 12,000, in 1760 between 15,000 and 20,000, in 1782 only 12,000. At the time of the first federal census in 1790 it was 18,038.

In order to appreciate the terrible nature of the scourge of smallpox which visited Boston so many times during the eighteenth century it is only necessary to glance at the following table:

Year	Population	No. Cases	No. Deaths
1721.....	11-12,000	5,080	850
1730.....	.....	4,000	500
1752.....	15,684	7,660	560
1764.....	15,500(?)	5,646	170
1776.....	6,573 civil + soldiery	5,202	57 (civil ?)
1778.....	13,500	2,243	61
1792.....	19,484	8,348	108

See law of 1776, chs. 7, 8

not in Boston, nor in other towns in the colony, was doubtless the occasion for the law which was passed in that year "to prevent the spreading of smallpox."<sup>15</sup> By this law it was made the duty of every person coming into any town of the province from any place in the neighboring colonies or provinces where smallpox or other malignant infectious distemper was prevailing, to notify one or more of the selectmen or the town-clerk under penalty of paying £20. The selectman might then warn such persons to remove, and if the warning were not heeded within two hours, a justice of the peace might issue a warrant to the constable to remove them and their goods by force. A second offense was to be punished by the forfeiture of £100. Nor were the inhabitants to entertain any person so warned to depart. Selectmen were authorized to appoint "meet persons" to attend at ferries and other places with power to examine suspected travelers and to hinder them until licensed by a justice of the peace of the county or the selectmen of the town.

The law was re-enacted three years later<sup>16</sup> with the addition of the requirement that heads of households should at once notify the selectmen of any case of smallpox in their families and should also hang out a red flag, to be displayed until the selectmen should pronounce the house thoroughly cleansed; but these provisions were inoperative "if more than twenty families were known to be visited with the smallpox at one and the same time." As so amended the law was repeatedly renewed until its repeal by the act of 1797.<sup>17</sup>

In 1751<sup>18</sup> an addition was made to the original law of 1701-2, giving to any justice of the peace the power to issue a warrant, upon representation of the selectmen, requiring the sheriff to secure suspected baggage or goods and to detain it until due inquiry should be made by the justice; if it appeared probable that the goods was infected with plague, smallpox, or other malignant contagious distemper a second warrant might be issued for its removal to a safe place and its disinfection. In case of opposition, force might be used; and if needful, upon a warrant, storage might be impressed.

The two parts of the act of 1701-2, marine and land quarantine, with the additions along each line were united in a single law in

<sup>15</sup> 1739, ch. 1; Webster, *Hist. Epid. Dis.*, I, p. 216.

<sup>17</sup> 1797, ch. 16.

<sup>16</sup> 1742-43, ch. 17.

<sup>18</sup> 1751-52, ch. 12.

1797.<sup>19</sup> This law continued unchanged the essential powers conferred by the earlier laws with regard to quarantine by land and by sea, isolation, and removal. There was added to these an important section in accord with the resolve of 1793 enabling each town, whenever it was thought necessary, to choose and appoint a health committee of from five to nine persons, or a health officer.<sup>20</sup>

Up to this time the fight against disease in Massachusetts had been almost exclusively against smallpox, as in New York against yellow fever. Except for the epidemics of "malignant fever" in 1647 and 1665, and a fever "brought from Barbadoes" in 1693,<sup>21</sup> which were probably yellow fever, the disease did not reach Boston until the widespread epidemic of 1798. In that year it spread over the entire country, and was brought to Boston in infected ships. There were about three hundred cases, at first chiefly along the wharves, but spreading thence to the better residence portions of the town and among those living "adjacent to the mill pond." These facts together with the comparative immunity of those living on higher ground led the majority of the doctors to accept the "non-contagious" theory of the origin of the disease and to find the causes in "marsh exhalation and human effluvia," or in "many green hides in a state of putrefaction stored near the wharves."<sup>22</sup> They recommended the construction of aqueducts and sewers, the removal of privies, graveyards, and nuisances, and "every attention and care to the preservation of cleanliness, indoors and out," also the cleansing of ships with lime; "less of ardent spirits, more of soap and water should be recommended and enjoined for ships' use."

Following the recommendations of the physicians the legislature in 1799 passed special acts for the towns of Boston<sup>23</sup> and Salem<sup>24</sup> creating boards of health in those towns with ample power over nuisances<sup>25</sup> as well as over quarantine. Under the sections on quarantine whenever in their judgment the safety of the inhabitants demanded the board of health was "required and empowered" to cause any vessel from any port "to perform quarantine under such restrictions, regulations, and qualifications as they may judge expedient." Within

<sup>19</sup> 1797, ch. 16.

<sup>20</sup> *Supra*, p. 44; *infra*, p. 52.

<sup>21</sup> *Memorial Hist. Bost.*, 4, p. 532.

<sup>22</sup> Brown, prize essay on *Yellow Fever in Boston*, 1801; *Report of Mass. Sanit. Commis.*

<sup>23</sup> 1799, ch. 10.

<sup>24</sup> 1799, ch. 14.

<sup>25</sup> *Infra*, p. 53.

a few months the legislature passed a general law in addition to that of 1797 giving similar power to the selectmen of any seaport town; or if there were a board of health or health officer in the town, to such board or officer. As so amended the law remained in force without alteration until the Revised Statutes were published in 1836.<sup>26</sup> At that time, while the wording was made more concise and was modeled after the wording of the act of 1816 for Boston, the essential powers of local boards over quarantine, both by sea and by land and including isolation and removal, were not altered, and they have continued unchanged through the General Statutes of 1860<sup>27</sup> and the Public Statutes of 1882.<sup>28</sup> Amendments since the latter date<sup>29</sup> have included diphtheria and scarlet fever with smallpox in the list for which a house-holder or physician must notify the board of health or the selectmen, and have included a requirement for disinfection in a manner approved by the board.

The quarantine provisions for the towns under special laws, later cities, have suffered so few changes that it will not be necessary to consider them apart from other powers of boards of health in towns and cities.

#### B. SPECIAL LEGISLATION AGAINST SMALLPOX: INOCULATION AND VACCINATION.

Quarantine, however, was not only the weapon used in the fight against smallpox. As early as 1721 "prodigious excitement" had been stirred up over a new practice, that of inoculation for smallpox. This practice had been introduced into England from the East by Lady Mary Wortley Montagu and was supposed to induce smallpox in a mild form, rendering the subject immune to further attacks; it differed from the modern practice of vaccination in that genuine smallpox, not the modified form known as cowpox, was introduced into the system. In April of 1721 smallpox was brought to Boston from Barbadoes.<sup>30</sup> In June of that year Cotton Mather, who had read in philosophical journals of the new practice in England, introduced it into Boston. Immediately a bitter controversy arose; the Mathers, father and son, Dr. Zabdiel Boylston, and many of the ministers were in favor;

<sup>26</sup> 1799, ch. 59. R. S., ch. 21, secs. 27-34.

<sup>28</sup> P. S., ch. 80, secs. 62-69.

<sup>27</sup> G. S., ch. 26, secs. 32-39.

<sup>29</sup> 1884, ch. 98; 1891, ch. 188; 1890, ch. 102.

<sup>30</sup> *Mass. Hist. Coll.*, 32, p. 168; Barry, *Hist. Mass. Bay*, 1, p. 114; *Mem. Hist. Boston*, 4, p. 525.



Dr. William Douglas and the few physicians of the town generally against. There was not only a war of pamphlets, but actual riots in which the lives of Cotton Mather and Dr. Boylston were threatened. The believers in inoculation persisted, however, in their experiments and the results were so successful that by 1752 even Dr. Douglas was converted.<sup>31</sup>

The matter first came before the legislature in January of 1764, when a temporary act for Boston was passed forbidding inoculation without a written permit from the selectmen "until thirty families are known to be visited with the said distemper at one time, unless the selectmen shall give public notice before that time that they have no hope to stop the progress of the said distemper."<sup>32</sup> Within a few days, by order of the council, an inoculating hospital was established at Point Shirley under the care of appointed physicians; later the barracks at Castle William were thrown open for the use of all physicians who wished to inoculate patients.

In the same year an addition was made to the act of 1701-2 authorizing selectmen of towns to appoint guards, who might be impressed by warrant, to prevent persons from entering or leaving a house where there was a case of smallpox.<sup>33</sup> Those suffering from smallpox, whether taken by inoculation or otherwise, might not leave their houses without a physician's certificate of proper cleansing. Persons from other places desiring inoculation in Boston must first obtain permission from the selectmen. Inoculating hospitals were forbidden in any town without the consent of the selectmen.<sup>34</sup>

During the War of Independence smallpox was spread by the army and again became epidemic. In view of the fact that "long experience" had shown the advantages of inoculation, the legislature passed a law<sup>35</sup> empowering one or more of the justices of the court of general sessions in any county to permit the establishment of inoculating hospitals in their county under such regulations and

<sup>31</sup> "A Forgotten Horror, Smallpox," *Essex Institute Hist. Coll.*, 35, p. 304; *Mass. Hist. Coll.*, 9, p. 276.

<sup>32</sup> 1763-64, ch. 17.

<sup>33</sup> 1764-65, ch. 12.

<sup>34</sup> At about this time or a little later the practice of inoculation was regulated or forbidden in many other states; South Carolina, 1738, ch. 651, forbidden within two miles of Charleston; 1764, ch. 930, forbidden entirely without permission from the governor; Virginia, 1769, ch. 26, forbidden without special license; Pennsylvania, 1823-24, ch. 5520, forbidden entirely without permit from board of health.

<sup>35</sup> 1776-77, ch. 7.



restrictions and in such place as they might order; inoculation at any place other than the county hospital so established was forbidden unless more than 20 families in a town were known to be visited with the smallpox.

At the same time in a special act for Boston<sup>36</sup> reciting that "whereas it appears that it has become impossible to prevent a general spread of smallpox in the town of Boston, and that it is of the utmost importance, considering the state of our public affairs, that the said distemper be carried through the said town with all possible dispatch" a general permission for inoculation was granted to Boston for a limited time. After the expiration of that time persons who had not had the disease were forbidden to enter the town until it was declared by the selectmen to be free from infection.

It may not be generally known that smallpox was among the difficulties with which the American armies had to contend during the War of the Revolution. The disease was constantly present in some portion of the continental armies. Very early in the war, 1775, Dr. Church, then surgeon-general, issued orders for the inoculation of the army in Massachusetts. In 1776 General Washington had inoculating hospitals for the troops fitted up at Morristown, N. J.; and at about the same time caused his wife to undergo inoculation. In February, 1777, he wrote to Congress that it was impossible to keep the smallpox from spreading through the whole army and that he had determined not only to inoculate the troops, but to order that recruits be inoculated at once upon enlistment so as to lose no time. The general inoculation of the army was undergone during the autumn of 1777 at Valley Forge.\*

In 1792, a year in which smallpox was most prevalent, a new law was enacted repealing the previous laws upon the subject of inoculation and authorizing inoculation hospitals to be erected by any town upon the vote of the inhabitants, such hospitals to be under the control of the selectmen; no one was to be inoculated except at such hospitals; in case of emergency the selectmen were given power to provide without a vote of the town a hospital for the reception of the sick and infected and might grant licenses for the inoculation there of all

<sup>36</sup> 1776-77, ch. 8.

\* Packard, *The History of Medicine in the United States*, pp. 264, 283, 315; Mumford, *A Narrative of Medicine in America*, p. 122.

persons supposed to have taken the infection; they might remove all sick or infected persons to these hospitals except at the risk of life, in which case the house in which the sick person remained was to be considered a hospital and under the regulation of the selectmen. Immediate notice of a case of smallpox must be given by householders to the selectmen and red flags displayed when required.<sup>37</sup>

The law of 1792 with regard to inoculation for smallpox was incorporated in the Revised Statutes in 1836 practically unchanged, although the practice itself had been superseded by a later scientific discovery, that of Jenner, with regard to vaccination. The new inoculation with vaccine virus had been introduced in Boston as early as 1800 by Dr. Waterhouse, and in 1802 experiments had been made under the direction of the Boston board of health to test the new theory.<sup>38</sup> These experiments were so successful that large numbers of persons were vaccinated and within a short time the disease was practically stamped out, at least in the epidemic form.

The first action of the legislature on the subject was in 1810<sup>39</sup> when a law was passed allowing towns to vote to provide for the vaccination of the inhabitants under the direction of the board of health, if there was one in the town, and, if not, under three persons elected for that purpose; and to provide funds for such general vaccination in the same manner as other town expenses were raised. Acting under this law some towns voted for re-vaccination of the inhabitants as often as every five years.<sup>40</sup> This law was so modified in the Revised Statutes that vaccination was not compulsory. But in 1855<sup>41</sup> Massachusetts took the most advanced stand ever taken by any of the states<sup>42</sup> and enacted a law which required parents or guardians to cause the vaccination of all children before they were two years old, and forbade the admission to the public schools of any child who had not been duly vaccinated. The selectmen of towns, mayors and aldermen of cities were to "enforce the vaccination of all the inhabitants" and to require re-vaccination whenever they

<sup>37</sup> 1792, ch. 58.

<sup>38</sup> *Mem. Hist. Bost.*, 4, pp. 545, 547.

<sup>39</sup> 1809, ch. 117.

<sup>40</sup> *Rep. Mass. Sanit. Commis.*

<sup>41</sup> 1855, ch. 414.

<sup>42</sup> Many European countries require vaccination. In England, since 1854, the last law requires it within six months of birth; Bavaria, 1807; Denmark, 1810; Sweden, 1874; several German states, 1818; Prussia, 1835; Roumania, 1874; Hungary, 1876; Servia, 1881.

judged the public health to require it; all employees of manufacturing companies, all inmates of almshouses, reform schools, lunatic asylums, and other places where the poor and the sick are received, of houses of correction, jails, prisons, of all institutions supported wholly or partly by the state were to be vaccinated at the expense of the corporation; towns and cities were to furnish the means of vaccination to such persons as were unable to pay. An amendment in 1894<sup>43</sup> exempted from the compulsory vaccination clause and admitted to school without vaccination a child certified to be an unfit subject by a physician. All vaccine institutions were placed under the supervision of the state board of health in 1894, but after a resolution of the legislature in 1902 calling for an investigation and report upon the quality of the vaccine matter produced and offered for sale in the state an amendment was passed empowering the state board to produce and distribute antitoxin and vaccine lymph.<sup>44</sup>

#### C. LOCAL BOARDS OF HEALTH AND NUISANCES.

According to the accepted theory of government in the colony of Massachusetts local affairs were to be left as far as possible to the management of the localities themselves. As far back as 1635 we find in the Records of the Colony of Massachusetts Bay in New England: "Whereas particular townes have many things which concerne only themselves, and the ordering of their own affairs . . . it is therefore ordered, that the freemen of every towne, or the major part of them, shall have power to . . . make such orders as may concerne the well ordering of their own townes, not repugnant to the laws and orders here established by the Generall Court." For nearly 60 years in a sparsely settled community further legislation regarding nuisances was found unnecessary.

The first law relating directly to nuisances was that of 1692<sup>45</sup> by which the selectmen and two justices of Boston, Salem, Charleston, and other market towns were given power to assign places for slaughter-houses, still-houses, and places for the trying of tallow and the currying of leather; such trades were forbidden at any other place than the one assigned. Already in 1710 "by reason of the growth and increase of said towns" the places assigned for the exercise of the

<sup>43</sup> 1894, ch. 515.

<sup>44</sup> 1894, ch. 355; 1902, Res. 107; 1903, ch. 480.

<sup>45</sup> 1692-93, ch. 23.

noxious trades had become inconvenient and offensive, and an additional act<sup>46</sup> conferred upon the same persons as before the power to assign places "from time to time," and to forbid and restrain the exercise of these trades in other places. In case any trade became a nuisance in the place originally assigned "by reason of offensive and ill stench or otherwise hurtful to the neighborhood" it was made lawful "for a court of general sessions of the peace within the county to cause inquiry to be made thereinto by a jury, and to suppress such nuisance by prohibiting and restraining the use thereof." In 1785 the act was extended to all towns<sup>47</sup> and further methods of enforcement included.<sup>48</sup>

A further act in 1801,<sup>49</sup> passed because "the laws now in force are inadequate to so speedy a removal of nuisances as the exigencies of the public require," added no new powers over nuisances to those already possessed by the towns, but is interesting from the method provided.<sup>50</sup> It is probable that sec. 12, chap. 21, of the Revised Statutes was intended to summarize the provisions of the law of 1801, but it is not very clear.

Meanwhile, in 1797<sup>51</sup> it was enacted that any town might choose a health committee of from five to nine persons, or appoint one person as health officer, with power to abate or order the abatement of all nuisances caused by filth of any kind, at the expense of the owner if they occurred on private property. After the yellow fever invasion in 1798 greater power to control nuisances was thought necessary in Boston and in February, 1799,<sup>52</sup> a board of health was created by special act for the town of Boston. This law conferred ample powers, but was almost immediately superseded by another (June, 1799)<sup>53</sup> creating a board of health to consist of one member elected from each ward.

It will be remembered<sup>54</sup> that the Boston physicians had leaned toward the opinion that the yellow fever of 1798 had been caused by noxious exhalations from putrid matter and had recommended a thorough cleansing of the city. By the terms of this act of 1799 it was made the duty of the board of health or of any member to examine into and abate all nuisances and sources of filth which might

<sup>46</sup> 1710-11, ch. 8.

<sup>47</sup> 1785, ch. 1.

<sup>48</sup> *Infra*, chapter on "Enforcement," p. 86.

<sup>49</sup> 1801, ch. 16.

<sup>50</sup> *Infra*, p. 90.

<sup>51</sup> 1797, ch. 16.

<sup>52</sup> 1798, ch. 47.

<sup>53</sup> 1799, ch. 10.

<sup>54</sup> *Supra*, p. 46.



be injurious to the health of the inhabitants; they were empowered to make such rules and regulations as appeared necessary for the removal of such nuisances; and two of them might obtain a warrant from a justice of the peace for forcible entry into suspected premises. The offering for sale or even the possession of putrid meat or fish was made a punishable offense and limitations were placed around the repacking of salted provisions; untanned hides were not to be stored anywhere within the town between the first of May and the first of December, but were forfeited if found in the town 24 hours after notice to remove. The board might appoint such scavengers and other officers as they thought proper. Lodging-house keepers were required to report to the board of health the names of seafaring men or other lodgers who became sick. The powers of the board included also provisions for quarantine, isolation, and removal. By similar laws boards of health were created in Salem,<sup>55</sup> Marblehead,<sup>56</sup> Plymouth,<sup>57</sup> and other towns.

Additional legislation increased the powers and duties of the board of Boston in certain particulars. Thus in 1803<sup>58</sup> if three physicians thought any part of Boston endangered by the prevalence of contagious sickness in that part, it was made the duty of the board of health to warn the inhabitants, and authority was given to close the streets and place guards if necessary. In 1809<sup>59</sup> the power was added to regulate funerals and burial grounds and to license undertakers.

In 1816<sup>60</sup> a new law superseded that of 1799, and became the basis of the present powers over health of the council of the city of Boston. A board of health was retained, one member to be elected from each ward. This board had power to make rules, regulations, and orders for preventing, removing, or destroying nuisances, sources of filth, and causes of sickness, but such rules must be published before going into effect; to examine suspected places in Boston, the islands, or vessels in the harbor, by force, under a search warrant from a justice of the peace, if necessary, and to abate when found; to isolate cases of contagious disease or remove them to Rainsford Island or elsewhere; to seize and destroy or remove unfit provisions; to make

<sup>55</sup> 1799, ch. 14.

<sup>56</sup> 1801, ch. 43.

<sup>57</sup> 1800, ch. 63.

<sup>58</sup> 1803, chs. 11, 125.

<sup>59</sup> 1809, chs. 10, 125.

<sup>60</sup> 1816, ch. 44.



rules and regulations as to clothing or other articles capable of conveying contagious disease; to establish and regulate quarantine, the rules of the board to extend to all persons on board ship, to visitors from shore, and to the cargo; to appoint a principal physician, an assistant physician, and such other officers and servants as were necessary, and fix their salaries; to have the care of Rainsford Island, the hospital, and other property; to appoint scavengers, superintendents of burying grounds, undertakers and fix their fees; to make rules for the burial of the dead and appoint places of burial; and to have any other powers conferred on the former board.

Upon the granting of the first charter of Boston as a city in 1822<sup>61</sup> all power and authority vested in the board of health of the town, in relation to quarantine and every other subject relating to health, were transferred to the city council "to be carried into execution by the appointment of health commissioners, or in such other manner as health, cleanliness and comfort and order may in their judgment require." From that time to this no board of health has been required by law in Boston, although the council established a board of health by ordinance in 1872.<sup>62</sup>

As other towns became cities through the enactment of special laws in the form of city charters similar provisions were made for the exercise of health powers as the council might see fit, so that the practice varied greatly in the different cities of the state. It was not until after the second epidemic of cholera in 1849<sup>63</sup> that a general law was passed transferring to city councils in all cities the powers and duties of boards of health in towns, with power to carry them into effect in any manner they might prescribe or through any person to whom they might delegate authority. In this law it was distinctly specified that the council might constitute either branch or any committee of their number a board of health for all purposes. Additional powers over nuisances were given and these additional powers were extended to towns by the law of 1855.<sup>64</sup>

In the same year an act was passed which recalls the acts of 1785 and 1801 in regard to slaughter-houses. Under its terms<sup>65</sup> boards of health of cities or towns were empowered to assign places for the

<sup>61</sup> 1822, ch. 110, sec. 17.

<sup>62</sup> *Mem. Hist. Boston*, 3, p. 279; *infra*, p. 63.

<sup>63</sup> 1849, ch. 211.

<sup>64</sup> 1855, ch. 369.

<sup>65</sup> 1855, ch. 391.

exercise of noxious trades and, in case of neglect or refusal to obey an order, to abate the nuisance by preventing the exercise of the trade with provision for appeal to a jury. This act differs from the earlier ones, however, in that by this later act the person aggrieved by the order of the board might apply for a jury of inquiry, whereas in the earlier acts it was the person complaining against the nuisance who applied for such a jury. The burden of proof has been thrown upon the creator of the nuisance.

Upon the approach of cholera again in 1866 the legislature passed two acts bearing upon health. By the first, persons aggrieved by the neglect of the board of health of a city or town to pass orders for the abatement of nuisances might appeal to the board of county commissioners for redress of their grievances.<sup>66</sup> The commissioners were given power to "hear and determine the matter of such appeal, and to make such decrees and exercise and perform the powers in such a case as a board of health may exercise and perform in a town." By the other act of that year<sup>67</sup> boards of health of cities or towns might appoint an agent to act for them in cases of emergency, or when the board could not conveniently be assembled; such an agent was to have all the authority of the board making the appointment, but must, within two days, report his actions to the board for approval.

In 1868 the powers of local boards of health were further increased by including the abatement, after notice and hearing, of nuisances caused by swamp lands upon petition of a person injured. If the board of health failed to act, the petitioner might apply to the Superior Court for the appointment of commissioners to determine the nuisance and abate it. In either case the damages and expenses caused by the abatement were to be assessed upon the property benefited similarly to those assessed for street improvements. This law, nominally a health law, partakes so largely of the nature of a law for economic improvements, that the sincerity of the health aspect is questionable.

More directly in the line of health was the law of 1871<sup>68</sup> forbidding the erection or use of a building for slaughtering in cities and towns of more than four thousand inhabitants without the written consent of the mayor and aldermen or selectmen; the law not to apply to

<sup>66</sup> 1866, ch. 211.

<sup>67</sup> 1866, ch. 271.

<sup>68</sup> 1871, ch. 167; extended in 1874 (1874, ch. 308) to all cities and towns.

buildings already in use. In another section the state board of health was given power, after notice and hearing, to order the cessation of offensive trades in any building where their conduct was injurious to health. An injunction might issue from the Supreme Court to carry out orders under either section.

Up to this time, since the first establishment of cities in the state by the Boston charter in 1822, there had been no legislative requirement for boards of health in cities, although such a requirement had been recommended by the Sanitary Commission in 1850. The law of 1849 had given all powers over health to city councils to be exercised as they saw fit. In 1877 a law was passed<sup>69</sup> requiring the submission to the voters of each city of the question whether or not a board of health should be established as provided in the act. For cities accepting the act a board of health was to be instituted consisting of three persons, none of whom should be members of the council, appointed by the mayor and aldermen, and the city physician, *ex officio*; all to receive compensation as fixed by the council. All powers and duties over health formerly vested in city councils as boards of health by statute and ordinances, including the appointment of subordinates, were transferred to the new boards of health so instituted; and they were given the additional power of making and enforcing regulations as to house drainage and sewer connections. This last-named power was extended to towns in 1889.<sup>70</sup> In 1879<sup>71</sup> it was enacted that the question of the acceptance of the law of 1877 must be presented to the voters at the next election upon the petition of fifty voters, and that in case of severe epidemic or danger to the public health the mayor and aldermen of cities having no board of health, upon petition of one hundred voters, might appoint such a board without the vote of the city. The law as thus amended was embodied in the Public Statutes of 1882.<sup>72</sup>

No further change of importance was made until 1895.<sup>73</sup> In that year a law was passed requiring in all cities, except Boston, the appointment by the mayor and aldermen of a board of health of three members, one each year, to serve for three years. There were no changes in the powers or duties conferred. The only addition to the powers

<sup>69</sup> 1877, ch. 133.

<sup>70</sup> 1889, ch. 108.

<sup>71</sup> 1879, ch. 114.

<sup>72</sup> P. S., ch. 80, secs. 8-17.

<sup>73</sup> 1895, ch. 332.

of local boards since that time has been the power to require a license from anyone about to engage in the business of slaughtering.<sup>74</sup>

Each town in Massachusetts has, therefore, had the right since 1797 to choose a board of health with powers over both quarantine and nuisances, which have been amplified from time to time. City councils in cities were to act as a board of health, or to appoint a board, at their discretion, first by provisions in city charters, after 1849 by general law; or, after 1877 by vote of the city, a special board of health, dissociated from the council, might be appointed; boards of health were not required by law until 1895 for all cities, and even then an exception was made in regard to Boston. Through all this legislation there runs that strong inclination to leave to the "particular townes . . . those things which concerne only themselves," which has always been characteristic of the people of Massachusetts.

#### D. THE STATE BOARD OF HEALTH.

In May of 1849, at about the time the cholera broke out for the second time in Massachusetts, a concurrent resolution of the two houses of the legislature enabled the governor to appoint a commission of three to prepare a report for a sanitary survey of the state including facts and suggestions. In a remarkably able report, published in 1850, the commission not only gave a plan for a sanitary survey of the state, but presented much matter which was in itself a sanitary survey, and made a series of recommendations for legislative and social action for the benefit of public health, which in many ways indicated the path which legislation has followed since that time, although the legislature has been very slow to act upon these foresighted recommendations.

Foremost among the recommendations of the commission was one for the creation of a "General Board of Health," for the entire state, which should "be charged with the execution of the laws relating to the enumeration, the vital statistics, and the public health," accompanied by cogent reasons for its adoption. It was nearly 20 years, however, before the legislature took action along this line.

In June of 1869<sup>75</sup> an act was passed for the creation of a state board of health to consist of seven persons appointed one each year

<sup>74</sup> 1901, ch. 134.

<sup>75</sup> 1869, ch. 420.



for a term of seven years by the governor, the members to be ineligible for reappointment. No member was to receive compensation except the secretary, an executive officer elected by the board. Meetings were to be held once in three months. The duties of the board were: to take cognizance of the interests of life and health among the citizens; to make sanitary investigations and inquiries as to the sanitary condition of the the people, the causes of disease, especially epidemics, the sources of mortality, the effects of localities, employments, conditions, and circumstances upon public health; to gather such information in these matters as they deemed proper for diffusion; to advise the government as to the location of public institutions; to examine and report on the effect of intoxicating liquors as a beverage on the industry, prosperity, happiness, health, and lives of citizens; and to make an annual report to the legislature embodying the results of these investigations with suggestions for further legislation.

As thus constituted the state board of health was a purely advisory body; its active participation in the administration of health matters began with a law in 1871<sup>76</sup> which gave it the power, in cities of more than 4,000 inhabitants, to order the cessation of offensive trades in buildings where they would be injurious to the public health, but such action could be taken only after notice to the parties interested and opportunity for a hearing. This power was extended in 1874 to include such buildings in all cities and towns.<sup>77</sup>

The state board for purely sanitary purposes continued until 1879 when it was abolished and its functions transferred to a new board called the State Board of Health, Lunacy, and Charity.<sup>78</sup> This board had no additional powers or duties except to make special investigations in case of smallpox or other contagious disease, when it was to act in consultation with the local authorities and was given co-ordinate powers with the local boards in every place.

The arrangement of combining the boards of health, lunacy, and charity continued until 1886, when a separate state board of health was again provided for, with the same organization as the original board in 1869, and with all the powers of the combination board over health matters.<sup>79</sup> Since that time the powers and duties of the

<sup>76</sup> 1871, ch. 167

<sup>77</sup> 1874, ch. 308.

<sup>78</sup> 1879, ch. 291.

<sup>79</sup> 1886, ch. 101.



state board have been gradually enlarged by the addition of many matters, not all of them bearing directly upon the public health. Among those more directly connected with public health have been: The supervision of all vaccine institutions,<sup>80</sup> later to produce and distribute pure vaccine lymph and antitoxins;<sup>81</sup> the power to require annual, later monthly, reports of vital statistics from town boards<sup>82</sup> and to prescribe their form; the duty of examining annually the sewage systems of cities and towns and of making a report to the legislature with recommendations;<sup>83</sup> the power of requesting cities to erect isolation hospitals for smallpox and other contagious disease with a penalty on the city for refusal or neglect to comply.<sup>84</sup> Among the powers and duties less strictly connected with health have been many connected with food; the investigation of the effect of sewage upon shell fish; the duties formerly performed by the state inspector and assayer of liquors; the consideration of the advisability of legislation for the regulation of undertakers; the investigation of factories as to conditions affecting safety as well as health of the employees; and monthly statements of the analysis of adulterated foods.

The most recent health legislation in Massachusetts looks toward the supervision by the state board of the health of the entire state without the necessary intervention of local boards, a most radical departure from the local government traditions of the state. By law in 1907<sup>85</sup> the legislature provided for the division of the entire commonwealth by the state board into 15 health districts. The governor and council are to appoint a state inspector of health in each district for a term of five years. The duties of these state inspectors are: to gather information concerning the sanitary condition of their districts, especially in regard to tuberculosis; to disseminate knowledge of the best methods of preventing that and other diseases; to take steps for their eradication after consultation with state and local authorities; to keep themselves informed as to the health of minors employed in factories, and if necessary to call the attention of parents and employers to cases of physical unfitness; to enforce the laws for ventilation and cleanliness

<sup>80</sup> 1894, ch. 355.

<sup>81</sup> 1903, ch. 480.

<sup>82</sup> 1897, ch. 428; 1903, ch. 305.

<sup>83</sup> 1901, ch. 104.

<sup>84</sup> 1901, ch. 171.

<sup>85</sup> 1907, ch. 537; cf. S. C. St. 1878, ch. 610; *infra*, Appendix A.

of factories and perform other duties formerly performed by the inspection department of the district police. All such sanitary inspectors are to be under the supervision of the state board of health, and are to perform such duties as the board shall impose upon them.

If the laws of the last few years are any indication of the trend of the legislation of the future, they seem to indicate a much more centralized system of health regulation and administration; a tendency toward the French system, in which there is a central department, with final authority over all municipal divisions. New York has already a state department with a commissioner at its head, Massachusetts has divided the state into health districts, it remains only to combine the two to have a system closely approaching that of France.

## PART II. ADMINISTRATION AS PROVIDED IN THE LAWS.

### CHAPTER 3.

#### ADMINISTRATIVE AUTHORITIES.

As has been already shown, legislative activity in the fight against disease has proceeded in the past mainly along two lines, and has included two spheres of activity, following the two theories as to the nature and origin of epidemic disease; first, acting on the theory of specific contagion, legislatures have attempted to exclude or limit diseases, chiefly smallpox, yellow fever, and cholera, by means of quarantine and isolation; secondly, upon the theory of atmospheric poison, they have attempted to improve conditions as to cleanliness, drainage, and sanitation, the chief means being the abatement of nuisances. To these two methods a third has latterly been added, that of scientific investigation followed by the hygienic education of the people.

Before considering historically the nature and distribution of the functions of administrative agencies in these various spheres, it will be well to discuss the sources of their authority, the methods of their selection, and their qualifications.

#### A. SOURCES OF AUTHORITY.

The source of authority for the creation of administrative bodies in health matters, as well as for the exercise of their powers, is ordinarily to be found in legislative action; however, in one state at least, Louisiana, the constitution requires the creation of a state board of health, and of local boards for every parish and municipality in the state, and in another (Maryland) the constitutions in force until 1867 gave the governor power to proclaim quarantine.<sup>2</sup> Frequently the question of the establishment of local boards, or of health officers, and their endowment with powers, has been left by statute to the determination of the local authorities, in which case the source of authority for such boards or officers is found in a city ordinance;<sup>3</sup> occasionally, it is left to the vote of the people of the locality, as in Massachusetts

<sup>1</sup> La. Const. 1898, Art. 296.

<sup>3</sup> Mass. St. 1849, ch. 211.

<sup>2</sup> Md. Consts. of 1776, 1781 1793.

towns in 1797 and cities in 1877.<sup>4</sup> On the other hand, in very early days municipal councils took action in regard to both quarantine<sup>5</sup> and nuisances<sup>6</sup> without a specific grant of power, relying either upon a general grant of power to do all that was necessary for the well-being of the inhabitants or upon some supposedly inherent power of municipalities.

## B. CONSTITUTION OF HEALTH AUTHORITIES.

1. *Methods of selection.*—There have been two methods commonly in use for the selection of boards of health and health officers, appointment and election. State health authorities, whether state boards of health or health officers, necessarily derive their authority from statute or constitution, and with few exceptions have been appointed by the governor, usually with the advice and consent of the senate or council.<sup>7</sup>

There has been less uniformity in the methods of selecting authorities for municipalities. Powers over health matters have frequently been granted specifically by city charter or special law to the regularly constituted local authorities who might themselves administer the law or, if the statute permitted, appoint either a part of their own number as a board or committee of health or other agents; such was the case in the charters of Boston in 1822 and Salem in 1836, and in the general law for all cities in Massachusetts in 1849.<sup>8</sup> In New York the charters of Schenectady in 1798, of Rochester in 1834, and of other cities and villages gave health powers to the city councils, and the law of 1850<sup>9</sup> to the council of New York City which was to be known as the board of health when acting in relation to health. In all these cases the council might appoint subordinate executive officers. In Massachusetts towns selectmen administered the health laws if neither a board of health nor a health officer had been elected;<sup>10</sup> and in New York

<sup>4</sup> Mass. St. 1797, ch. 16; 1877, ch. 133; see also 1855, ch. 391; 1877, ch. 133; 1890, ch. 74; 1899, ch. 184.

<sup>5</sup> New York City, 1680, *supra*, p. 3; Salem, Mass., 1678, *supra*, p. 41.

<sup>6</sup> New York City, 1670; *infra*, p. 77.

<sup>7</sup> State Board, Mass. St. 1869, ch. 420; State Board, N. Y. St. 1880, ch. 322; health officer of port of New York since the beginning; district officers, Mass. St. 1907, ch. 537; cf. State Board, S. C. St. 1878, ch. 610; *infra*, p. 124; 21 Cyc. 382.

<sup>8</sup> Mass. St. 1822, ch. 110; 1840, ch. 211.

<sup>10</sup> Mass. St. 1797, ch. 16.

<sup>9</sup> N. Y. St. 1850, ch. 275

towns supervisors have been required to act with the justices of the peace as a board of health since 1850.<sup>11</sup>

More commonly, however, especially in later years, the statutes have required that the administration of health matters should be in the hands of persons especially chosen for that purpose and distinct from the city council or regular governing body; either a local board of health, a health officer, or both. The authorizing statute has usually provided for the appointment of such persons by the regular local authorities, as in Massachusetts by the laws of 1877 and 1895 for cities,<sup>12</sup> and in New York for all cities and villages with exceptions named in the statutes, since 1850;<sup>13</sup> although in the Massachusetts law for towns they may be elected by the voters<sup>14</sup> and by the law of 1877 for cities a vote of the citizens was necessary for the adoption of the law.

The administration of health matters by city councils has never met with the same confidence on the part of the people as when these functions have been performed by those chosen especially for the purpose; even a board created and appointed by the council has been preferred to the council itself. It has already been shown into what depths of degradation New York City health affairs fell under the political guidance of the city council.<sup>15</sup> In Boston affairs were never quite so bad, but "as the city increased in size, many important questions affecting public health were constantly arising, questions which the aldermen were not competent to deal with, but they were slow to recognize their incompetency and were quick to take offense at the advice tendered by their medical assistants."<sup>16</sup> Such a state of affairs continued until the aldermen had well-nigh lost the respect and confidence of the community. Finally, in 1872, after a badly managed epidemic of smallpox, the force of public opinion became so strong that they passed an ordinance creating a board of health and delegated to it all necessary powers.

In New York since 1846, the constitution has provided for the election, or appointment by the regular authorities, of all county, city, town, and village officers. This clause of the constitution stood in the way of placing the appointment of the New York City board

<sup>11</sup> N. Y. St. 1850, ch. 324.

<sup>12</sup> Mass. St. 1877, ch. 133; 1895, ch. 322.

<sup>13</sup> N. Y. St. 1850, ch. 324; N. Y. St. 1805, ch. 31, for New York City.

<sup>14</sup> Mass. St. 1707, ch. 16.

<sup>16</sup> *Memorial History of Boston*, 3, p. 279.

<sup>15</sup> *Supra*, p. 24.



in the hands of competent persons after the city council had proved themselves unworthy. The consequent formation of the Metropolitan Health District has already been described.<sup>17</sup> Because it was held contrary to the same clause in the constitution, the section of the New York law of 1885,<sup>18</sup> providing for the appointment of local boards and health officers by the county judges in case the local authorities failed to act, was declared unconstitutional.<sup>19</sup> It is doubtful whether the provision of the law of 1903<sup>20</sup> for the appointment of local health officers by the state commissioner of health upon nomination of the local board would stand the same test, although it is possible that the courts might consider the constitutional provision saved by the nomination of the local board.

Health laws have frequently imposed duties upon persons, other than the regularly constituted health officers, sometimes upon officers already serving the state in other capacities. In early quarantine laws pilots were required to warn incoming vessels of the law, later with a copy, upon pain of forfeiting their "branches;" military officers of the forts or castles commanding the harbor were required to make inquiries, to stop infected vessels, and to prevent their approaching the wharves;<sup>21</sup> the mayor of a city is frequently *ex officio* a member of the board of health; in both New York and Massachusetts, in early days, administrative duties in regard to health were laid upon justices of the peace, in New York up to the present time; in Massachusetts county commissioners have the powers of a board of health when appealed to by a person aggrieved at the neglect of a local board to abate nuisances;<sup>22</sup> and in New York during the existence of the state board of health, both the secretary of state and the state surveyor were *ex-officio* members.<sup>23</sup>

<sup>17</sup> *Supra*, p. 25. As to other health districts, see: Pa. St. 17 Mar. 1806, for a quarantine district including Philadelphia; La. St. 1848, p. 110, for a quarantine district including New Orleans; Ill. St. 29 May 1880, for the sanitary drainage district including Chicago; Cal. St. 1891, ch. 161; S. C. St. 1878, ch. 610, and 1892, ch. 16, for power of the state board to create sanitary districts; also Mass. St. 1907, ch. 537.

<sup>18</sup> N. Y. St. 1885, ch. 270, continued in 1893, ch. 661, and 1903, ch. 383.

<sup>19</sup> P. v. Houghton, 182 N. Y. 301; cf. Philadelphia board appointed by the judges, Pa. St. 1850, ch. 305.

<sup>20</sup> N. Y. St. 1903, ch. 383.

<sup>21</sup> Commander of Castle William, Mass. St. 1717-18, ch. 14; pilots, Mass. St. 1799, ch. 10; N. Y. St. since 1784, ch. 57; pilots and commander of the fort, S. C. St. 1721, ch. 438; 1759, ch. 881; collector of the customs, Pa. St. 1742-43, ch. 354.

<sup>22</sup> Mass. St. 1866, ch. 211.

<sup>23</sup> N. Y. St. 1880, ch. 322.

2. *Qualifications of members of boards of health and health officers.*—

Among the qualifications required of members of boards of health and health officers, citizenship and residence would naturally be expected, although, as a matter of fact, they are mentioned in very few laws.<sup>24</sup>

The requirement that there should be one or more physicians on a board of health, recommended both by the Massachusetts Sanitary Commission in 1850 and by the Public Health Association in 1875, would seem to be both reasonable and essential, and yet until after the middle of the nineteenth century was unusual.<sup>25</sup> In New York City, so long as the commissioners of health were members of the board, i. e., until 1823, there was at least a fair proportion of physicians, but after 1823 no physician member was required and the board rapidly degenerated from a professional board with public interests at heart to a mere cog in the political machine. The feeling for the necessity of physicians on the board and for the separation of the board from local politics was shown in the Metropolitan Health Act by which three of the four commissioners must be physicians<sup>26</sup> and no member might hold other political office. Upon the disintegration of the Metropolitan Board and the re-establishment of the city board under the charter of 1870 two of the members were required to be physicians, and since 1873<sup>27</sup> at least one. The requirement has been much more common that executive officers should be physicians; the health officer of the port of New York has been a physician by requirement of the law since 1798; the resident physician since 1799; and the third commissioner from 1823 to 1850. Since 1866 the sanitary superintendent must be a physician of 10 years' practice and since 1882 a large proportion of the sanitary inspectors have been required to be physicians.<sup>28</sup> No qualifications for members of local boards

<sup>24</sup> N. Y. St. 1863, ch. 358; P. v. Platt, 117 N. Y. 159; for sanitary superintendent, New York City, N. Y. St. 1873, ch. 335; 1897, ch. 378.

<sup>25</sup> In Chicago no physician was required upon the first board of health from its establishment in 1837 to its abolition in 1860. There was no board of health from 1860-67, all health matters being under the police department. From 1867-76 three of the six members of the board were required to be physicians. In 1876 the board was abolished by ordinance and a department of health created with a commissioner of health at its head. There was no requirement that the commissioner should be a physician until 1905. (Report of City Board Health, Chicago, 1867, 1; Kirkland, *History of Chicago*, City Ordinances).

<sup>26</sup> N. Y. St. 1866, ch. 74; also the sanitary superintendent.

<sup>27</sup> N. Y. St. 1870, ch. 137; 1873, ch. 335.

<sup>28</sup> N. Y. St. 1882, ch. 410; 10 out of 15.

of health were prescribed by the law of 1850, but by the amendment of 1881 members of boards of health of cities might not be appointed from among the members of the city council, nor after 1885, in villages, from the trustees; since 1885 one member of a city board must be a physician. That local health officers should be physicians has always been required in New York.<sup>29</sup>

In Massachusetts no requirements of any kind were made in the laws for local boards or officers until 1877<sup>30</sup> when, in cities which voted to have a board of health, the members might not be appointed from the city council, and the city physician was *ex officio* a member of the board; since 1895<sup>31</sup> one of the three members of all city boards must be a physician.

In New York the law establishing the state board of health required that two of the three members appointed by the governor should be physicians and since 1901<sup>32</sup> the state commissioner, at the head of the Department of Health, must be a physician of at least 10 years' practice. The Massachusetts law demanded no special qualifications for the state board and it has been held that women are eligible.<sup>33</sup> Under the law of 1907<sup>34</sup> the district state inspectors of health must be "learned in the science of medicine and hygiene."<sup>35</sup>

3. *Organization*.—Where control over health matters is in the hands of a board of health appointed by, or agents of, the regular local officers of a municipality, the incorporation of such a board is superfluous and has not been customary; although some of the incidents of incorporation, as the power to bring suit in the name of the board rather than of an individual,<sup>36</sup> and the liability of the board as an entity rather than that of the individuals composing it, have sometimes been expressly conferred by the statutes authorizing the board.<sup>37</sup> Unless such provisions are made in the statute the New York courts have held that a board of health could not be made a party to a suit.<sup>38</sup> In municipalities where the mayor and council act

<sup>29</sup> N. Y. St. 1832, ch. 333; 1850, ch. 324; 1881, ch. 431; 1885, ch. 270; 1903, ch. 383.

<sup>30</sup> Mass. St. 1877, ch. 133.

<sup>33</sup> Opinion of justices, 136 Mass. 578.

<sup>31</sup> Mass. St. 1895, ch. 332.

<sup>34</sup> Mass. St. 1907, ch. 537.

<sup>32</sup> N. Y. St. 1880, ch. 322; 1901, ch. 29.

<sup>35</sup> In Louisiana the law establishing a state board in 1855 (St. 1855, No. 336) required that members should be appointed "with reference to their known zeal in favor of a quarantine system."

<sup>36</sup> For New York City, N. Y. St. 1823, ch. 71, sec. 31; Gould v. Rochester, 105 N. Y. 46, 1887.

<sup>37</sup> Metropolitan Board, N. Y. St. 1866, ch. 74; 1866, ch. 686; 1867, ch. 956, secs. 6-8.

as a board of health they do so as representatives of the incorporated municipality, but where special districts have been created the necessity has occasionally been felt for the incorporation of the board regulating health matters within the district; this has been the case in Pennsylvania and Louisiana,<sup>39</sup> although in New York, upon the creation of the Metropolitan Health District there was no incorporation.

Probably the right to organize by choosing officers, a president and secretary, and to pass by-laws for the government of the board would be inherent in boards of health without specific statutory authority, but where boards have been created by statute it has been customary to specify that such boards might complete their organization by the election of officers and the passage of by-laws<sup>40</sup> although in some cases the presiding officer is designated in the statute or is such *ex officio* by virtue of other office, as where the mayor presides,<sup>41</sup> and always the secretary is appointed by the board, but is not always himself a member.<sup>42</sup>

The frequency of meetings to be held by the board has not uncommonly been determined by statute; boards having charge of quarantine in particular were often required to meet daily during the season when the danger of an invasion of yellow fever was greatest. The earliest laws in New York required the commissioners of health to meet "from time to time,"<sup>43</sup> but after 1811 daily meetings were required from the end of May to the first of October.<sup>44</sup> The tendency in later laws has been to require stated meetings less frequently and to leave to the discretion of the board the necessity for special meetings. Laws establishing state boards of health have frequently specified that they should meet once in three months.<sup>45</sup> In New York meetings

<sup>38</sup> *Gardner v. Bd.*, 10 N. Y. 409, 1852; *P. v. Supervisors Monroe Co.*, 18 Barb. 567; *Green Island Bd. v. Magill*, 17 N. Y. App. Div. 249; *Bd. Health v. Valentine*, 11 N. Y. Supp. 112.

<sup>39</sup> Pa. St. 17 Mar. 1806; 29 Jan. 1818; La. St. 17 Mar. 1818; 17 Feb. 1821, sec. 11; 1855, No. 336.

<sup>40</sup> Boston, Mass. St. 1798, ch. 47; 1799, ch. 10; other cities, 1877, ch. 133; Metropolitan Board, N. Y. St. 1866, ch. 74; State Board, N. Y. St. 1880, ch. 322.

<sup>41</sup> Mayor, New York City, N. Y. St. 1850, ch. 275; president to be the non-physician commissioner, N. Y. St. 1873, ch. 335; Philadelphia, Director of Public Safety to preside, Pa. St. 1885, ch. 33.

<sup>42</sup> Secretary a member or otherwise, New York State Board, N. Y. St. 1880, ch. 322; secretary a member after election by the State Board, Mass. St. 1869, ch. 420.

<sup>43</sup> N. Y. St. 1797, ch. 16; 1798, ch. 65.

<sup>44</sup> N. Y. St. 1811, ch. 175; daily, June 1 to Oct. 1, Pa. St. 1806; daily, June 1 to Nov. 1, La. St. 17 Feb. 1841.

<sup>45</sup> N. Y. St. 1880, ch. 322; Mass. St. 1869, ch. 420; La. St. 1898, No. 192; once in six months, Pa. St. 1885, ch. 37.



of local boards may be requested by the state board<sup>46</sup> and a violation of any instruction of the state board has been made a misdemeanor.

4. *Tenure*.—The tenure of office of members of appointed boards of health was not commonly fixed in early statutes. Where boards of health or officers have been elected, as in Massachusetts towns, the elections have usually been annual. When the tenure first began to be fixed in the statutes the tendency was for a short term, but the tendency in later laws has been to secure greater experience by lengthening the term.<sup>47</sup> Continuity has been frequently secured by providing for the expiration of one or more terms each year; this has been done in the Massachusetts state board since its inception; the terms are long, seven years, but so arranged that one member leaves the board each year and may not be reappointed; the same arrangement has been followed in Massachusetts cities since 1877 and in New York cities since 1881; it was also followed in the law for the Metropolitan Board in New York.

5. *Compensation*.—To secure the greatest efficiency in the administration of the health laws it is necessary not only to require professional training from one or more members of the board or their executive officer, but to employ the whole time and service of one or more persons; this involves the question of salaries. In the very early days of health legislation rewards were offered by means of *qui tam* actions to persons who would prosecute offenders, but soon the necessity for regular executive officers was felt, and such executive officers have always received compensation for their services.

The practice with regard to salaries for members of boards of health has varied greatly. Members of state boards have not ordinarily received compensation beyond their expenses, except the secretary, who might or might not be a member of the board.<sup>48</sup> The New York state commissioner of health receives a salary of \$4,500; that of the secretary of the Massachusetts state board was increased in 1907 to \$5,000. As to municipal boards, where the members of a city council act as a board of health it would not be expected that they should re-

<sup>46</sup> N. Y. St. 1885, ch. 270.

<sup>47</sup> New York Metropolitan Board, 4 years; New York City Board, 1870, 5 years, 1882, 6 years; New York State Board, 3 years, State Commissioner, 4 years; Mass. cities, 1877, 2 years, 1805, 3 years; New York local boards, 1850, 1 year, cities, 1881, 3 years.

<sup>48</sup> N. Y. St. 1880, ch. 322; Mass. St. 1869, ch. 420; 1886, ch. 101.



ceive salaries, nor where other officials are *ex-officio* members, although an exception was found to the latter rule in the case of the New York Metropolitan Police Commissioners who received extra compensation for their services upon the Metropolitan Board of Health.<sup>49</sup> The salaries of the Metropolitan Board and of the New York City board since 1870 have been fixed by statute;<sup>50</sup> in other cities of New York there is no compensation. In Massachusetts cities the amount is left to the city council to determine.<sup>51</sup>

In general, if the policy of the law has been to have a working board, giving full time, that board is small in number and the members receive salaries; if, on the contrary, the functions of the board are largely of an advisory or investigative nature, the numbers are greater and the members serve without compensation, the executive duties falling upon a paid secretary; the honor of appointment, professional interest, or altruistic motives are supposed to be sufficient inducement.<sup>52</sup>

Quarantine officers, in the earlier New York statutes, were authorized to demand fees, fixed in the statute, from all vessels examined, and, in addition, received a fixed salary as physicians to the marine hospital; but after the decision of the United States Supreme Court, holding the passenger fees unconstitutional,<sup>53</sup> the demand for fees for inspecting vessels was dropped along with the passenger tax, and the health officer of the port was given a fixed salary by the law of 1850.<sup>54</sup>

### C. NATURE OF FUNCTIONS EXERCISED.

The chief functions to be expected of boards of health would, of course, be administrative, the supervision of the execution of the health laws. The principle of separation of powers has not been

<sup>49</sup> N. Y. St. 1866, ch. 74.

<sup>52</sup> Mass. St. 1877, ch. 133.

<sup>50</sup> N. Y. St. 1897, ch. 378, sec. 1194.

<sup>51</sup> The Louisiana law of February 17, 1821, said: "The duties required from the board of health, being those inculcated by precepts of universal benevolence, as well as a regard for the particular welfare of the community of which they are members, the people of the state annex no pecuniary compensation to the performance of those duties; but in every year in which the state shall have escaped the visitation of an infectious disease, the Governor shall, at the annual meeting of the General Assembly of the state, communicate to them by message the names of the president and members of the board of health and of the physicians, under whose care, by the aid of Divine Providence, such exemption from infectious disease shall have been enjoyed."

<sup>53</sup> Passenger Cases, 48 U. S. 283.

<sup>54</sup> N. Y. St. 1850, ch. 275.

carried out, however, with absolute strictness, for besides executive powers, others, both quasi-legislative and quasi-judicial, have been conferred, subsidiary to the administration of the laws.

1. *Administrative*.—Among the administrative powers commonly conferred has been the appointment of subordinate officers and the fixing of their compensation. In all laws for the port of New York since 1811<sup>55</sup> the health officer has been empowered to appoint one or more assistants. The appointment of health wardens was by the New York law of 1820 given to the mayor and council, but in 1823<sup>56</sup> was put in the hands of the board of health, as also the appointment of consulting physicians. In 1850 the board of health, i. e., the mayor and council, appointed visiting, hospital, and consulting physicians, the city inspector all other subordinates: health wardens, inspectors, and the like. In 1866 the appointment of all subordinates was given to the Metropolitan Board, and since 1870 the power has been exercised by the city board of health.<sup>57</sup> Local boards in New York always appointed the local health officer as well as other assistants until 1903 but since that date the local health officers have been appointed by the state commissioner on nomination of the local board.<sup>58</sup>

In Massachusetts since 1739<sup>59</sup> selectmen of towns might appoint persons to execute their orders, and whenever there have been boards of health in cities these boards might appoint subordinate officers and other employees.<sup>60</sup>

The making of by-laws and the issuing of orders and directions are the natural means by which administrative bodies act and are essential powers for a board of health which is a truly executive agent. The power to make and execute orders is found in nearly all the laws establishing such boards for municipalities; almost the only exception is found in the general New York law of 1850,<sup>61</sup> which, while conferring power to make and publish regulations, did not mention orders; after an interpretation of this law in 1854 by the Supreme Court in *Reed v. P.*,<sup>62</sup> which held that a board of health was *ultra vires* in making special orders, the defect was remedied by the

<sup>55</sup> N. Y. St. 1811, ch. 175; 1820, ch. 220; 1846, ch. 300, etc.

<sup>56</sup> N. Y. St. 1823, ch. 71.

<sup>57</sup> N. Y. St. 1850, ch. 275; 1866, ch. 74; 1870, ch. 137.

<sup>60</sup> Mass. 1877, ch. 133; *Com. v. Swasey*, 133 Mass. 538.

<sup>58</sup> N. Y. St. 1903, ch. 383; *supra*, p. 64.

<sup>59</sup> Mass. St. 1739-40, ch. 1.

<sup>61</sup> N. Y. St. 1850, ch. 324.

amendment of 1867<sup>63</sup> which, with all later laws, included "orders." In the law establishing the Metropolitan Board such orders were made presumptive evidence of the facts referred to in the order. Massachusetts laws have all included the making of orders among the powers of boards of health or health officers. The execution of orders of boards of health has usually been intrusted to subordinates appointed by the board, although it is also often required of police officers, sheriffs, or constables. The latter has been true of warrants issued by the health officer of the port of New York; of warrants issued by boards of health under the act of 1850;<sup>64</sup> and of orders of the Metropolitan Board<sup>65</sup> and of later boards for New York City.

There is a diversity of opinion as to whether the power to make orders includes the power to issue licenses or not. In Massachusetts the affirmative ground is taken, in New York, the negative.<sup>66</sup>

Other executive powers usually conferred on boards of health have been to visit and examine vessels, buildings, and other places, to demand information and reports from physicians, and sometimes to make contracts.<sup>67</sup>

2. *Quasi-legislative*.—Partaking rather of the nature of legislation than execution is the power to make rules and regulations with the force of ordinances. Such regulations differ from administrative orders in that they are of general application, act equally upon all, and the power is usually accompanied by a requirement for publication; executive orders, on the other hand, are of special application to special cases, and no publication, but a notice of the order, is required. In some early New York laws where no publication of rules and regulations was required the statute required notice to the governor and his approval before the regulation became effective.<sup>68</sup>

Where the regular municipal authorities act as a board of health the power to make regulations with the force of ordinances might be implied under a general grant over health matters, but has frequently been given specifically to the mayor and council when acting as a board of health, as in New York City in 1850,<sup>69</sup> although at that time

<sup>63</sup> 1 Park Cr. 481.

<sup>64</sup> N. Y. St. 1850, ch. 324; 1863, ch. 358.

<sup>65</sup> N. Y. St. 1867, ch. 790.

<sup>65</sup> N. Y. St. 1866, ch. 74.

<sup>66</sup> *Quincy v. Kennard*, 151 Mass. 563, 1890; *Flushing v. Carraher*, 87 Hun 63, 1895.

<sup>67</sup> N. Y. St. 1866, ch. 74.

<sup>69</sup> N. Y. St. 1850, ch. 275.

<sup>68</sup> N. Y. St. 1796, ch. 38; 1797, ch. 16.

the power to issue orders was granted not only to the board but to the city inspectors also; where, however, a board of health has been created by statute the power to make rules and regulations with penalties attached for disobedience must be included in the statute,<sup>70</sup> and limitations as to the punishment have frequently been imposed in the statute itself. In New York since 1895<sup>71</sup> local boards have been able to impose penalties within certain limits for violation of their regulations. In New York City such ordinances comprising the "Sanitary Code," by which name the regulations of the Metropolitan Board of 1866 were known, were declared to be binding by statute in 1880, thus being given the force of statutes.<sup>72</sup> In Massachusetts town boards of health did not have quasi-legislative powers by general statute except for quarantine until the passage of the Revised Statutes in 1836; although in Boston the board of health had power from its first establishment in 1799<sup>73</sup> to make and execute rules, regulations, and orders for both quarantine and nuisances.<sup>74</sup>

State boards were primarily created for purposes of investigation; the power to make regulations came later. In New York the state board might make regulations first in 1885<sup>75</sup> for the protection of the water supply, although such regulations became effective only after the approval of the county judge or<sup>76</sup> a judge of the Supreme Court. The power to reverse or modify a regulation of a local board<sup>77</sup> would also be classed among the quasi-legislative powers. This power of the state board was transferred along with all other powers to the state commissioner in 1901.<sup>78</sup> It is a question whether the placing of so important a quasi-legislative power in the hands of an appointed official would stand a test in court or not. It is similar to the veto power of the governor.

In Massachusetts the state board might order the cessation of noxious trades in cities of more than 4,000 population after notice

<sup>70</sup> *Polinsky v. P.*, 73 N. Y. 65; *Taunton v. Taylor*, 116 Mass. 254; *infra*, p. 86.

<sup>71</sup> N. Y. St. 1895, ch. 203.

<sup>72</sup> N. Y. St. 1880, ch. 135; 1882, ch. 410, sec. 575; 1897, ch. 378, sec. 1172.

<sup>73</sup> Mass. St. 1799, ch. 10.

<sup>74</sup> For further legislative powers, see N. Y. St. 1850, ch. 324, for local boards; 1797, ch. 16, for New York City; 1798, ch. 65, and 1801, ch. 86, for commissioners of health; 1805, ch. 31, for mayor and council; also Mass. St. 1877, ch. 133.

<sup>75</sup> N. Y. St. 1885, ch. 543.

<sup>77</sup> N. Y. St. 1893, ch. 661.

<sup>76</sup> N. Y. St. 1888, ch. 52.

<sup>78</sup> N. Y. St. 1901, ch. 29.



and hearing,<sup>79</sup> and since 1879<sup>80</sup> has had "co-ordinate powers with local boards in every locality" in times of epidemic disease.

3. *Quasi-judicial*.—Certain powers of still another kind, quasi-judicial, have sometimes been granted to boards of health.<sup>81</sup> The first among the quasi-judicial functions to be granted was the power to administer oaths, granted to the health officer of the port of New York in 1796,<sup>82</sup> and to the selectmen of towns in Massachusetts for quarantine purposes in 1749.<sup>83</sup> The power to issue warrants was given in New York by the temporary cholera act of 1832 and was continued among the powers of all local boards upon the revival of that act in 1850; it was also granted to the health officer of the port in 1863, and to the Metropolitan Board in 1866.<sup>84</sup> In 1888 local boards were given the same power to issue subpoenas and compel the attendance of witnesses as justices of the peace, and the state board the same as judges of the Supreme Court.<sup>85</sup> The power to grant a hearing after notice to show cause was given to the board of New York City in 1850<sup>86</sup> and continued to the Metropolitan Board; in the latter instance the constitutionality of the act was attacked on the ground that it conferred judicial powers upon the Metropolitan Board whose members were appointed and not elected officers, but the court held in *Met. Bd. v. Heister*<sup>87</sup> that the power was not properly judicial and in no way prevented a final determination by a court.

In Massachusetts, aside from the administering of oaths referred to above, no quasi-judicial powers were given to boards of health until 1868<sup>88</sup> when local boards were empowered to abate nuisances caused by swamps only after notice and hearing. In 1871<sup>89</sup> the state board was empowered to forbid the exercise of noxious trades in cities or towns of more than four thousand inhabitants after notice and hearing. The Massachusetts Supreme Court, following a different opinion from that of the New York courts, has held that determinations of a board of health are judicial in character, even without a hearing;<sup>90</sup>

<sup>79</sup> Mass. St. 1871, ch. 167.

<sup>82</sup> *Infra*, p. 93.

<sup>83</sup> Mass. St. 1749-50, ch. 6.

<sup>80</sup> Mass. St. 1879, ch. 291.

<sup>81</sup> N. Y. St. 1796, ch. 38.

<sup>84</sup> N. Y. St. 1832, ch. 333; 1850, ch. 324; 1863, ch. 358; 1866, ch. 74.

<sup>85</sup> N. Y. St. 1888, ch. 146.

<sup>88</sup> Mass. St. 1868, ch. 160.

<sup>86</sup> N. Y. St. 1850, ch. 275.

<sup>89</sup> Mass. St. 1871, ch. 167.

<sup>87</sup> 37 N. Y. 661, 1868.

<sup>90</sup> *Salem v. Eastern R. R. Co.*, 98 Mass. 431; *Taunton v. Taylor*, 116 Mass. 254; *Peebles v. Boston*, 131 Mass. 197.



following this line of opinion in Massachusetts, provision is always made for an appeal from the decisions of a board of health, either expressly in the act, or it will be read into the statute by the courts.

#### D. DISTRIBUTION OF FUNCTIONS.

The spheres of activity of the various authorities in charge of the administration of the health laws extends over a variety of subjects of which those relating most closely to health and disease may be classed under the heads of quarantine, nuisances, and investigation, the last including the acquisition and distribution of sanitary knowledge, and in recent years the manufacture and distribution of preventive and curative sera and vaccines.

1. *Quarantine*.—Maritime quarantine for important ports has been enforced by central or state authorities in this country from the earliest times. The earliest instances of quarantine were all by "orders;" in Massachusetts in 1647 and 1665 by orders of the General Court; in 1647 by an order in council in New Netherlands; in 1693 by a "resolution of the inhabitants" in Charleston, S. C.; and in 1712 by orders in council in New York. All of the earliest laws on the subject, those of 1700 in Massachusetts and Pennsylvania, and of 1712 in South Carolina, provided for their administration by central authorities. From the first law for the port of New York in 1755, providing for a proclamation of quarantine to be issued by the governor and enforced by his appointees, to the present time the execution of the quarantine laws has been by an officer appointed by the governor; for smaller ports, aside from Albany and Hudson, quarantine was to be enforced by two justices, although, as the sea coast of the state is of limited extent and largely included in the harbor of New York, no vessel was likely to reach the smaller ports without passing through New York harbor and being subjected to the quarantine restrictions of the port. The probability was, therefore, remote of quarantine being declared for other than merely local protection. The practice of New York in regard to quarantine was the usual one in states having a port of pre-eminent importance, as Philadelphia in Pennsylvania, Baltimore in Maryland, Charleston in South Carolina, and New Orleans in Louisiana. In Massachusetts and other New England states, on the contrary, with an extended sea coast, with

several ports of almost equal importance, with little danger arising from the importation of disease, especially yellow fever, from foreign sources, and with a strong leaning toward local self-government, the enforcement of maritime quarantine, after the veto of the first general law, has always been left in the hands of the local authorities, in the earliest laws justices of the peace, a little later selectmen of towns or local boards of health; although even in Massachusetts in the early laws the governor was to be notified of the measures taken and was empowered to "take such further steps as are necessary."<sup>91</sup>

Land or local quarantine has been usually and until recent times entirely in the hands of the local authorities. In Massachusetts it has always been proclaimed and enforced by the selectmen of towns or local boards;<sup>92</sup> in New York in early days, aside from the cities of Albany, Hudson, and New York, by two justices of the peace,<sup>93</sup> later by local boards of health.<sup>94</sup> In New York City the commissioners of health since 1798, the mayor and board of health since 1805, have had the power to order any vessel, suspected of harboring infectious disease and found at the wharves, to the quarantine anchorage or other place.<sup>95</sup> The board of health has had power to isolate or remove infected persons and things and to fence off an infected part of the city since 1823;<sup>96</sup> also to proclaim local quarantine against an infected place and prohibit or regulate intercourse.<sup>97</sup>

There has been a strong tendency in recent years toward centralization of power, especially with regard to local quarantine; this has been true in New York since 1893<sup>98</sup> and is seen even in Massachusetts, the stronghold of local government.<sup>99</sup>

Quarantine laws in New York have usually been given in great detail, so that there has been little need for regulations to be made by the administrative authority; the tendency, however, since the middle

<sup>91</sup> Mass. St. 1701-2, ch. 9; cf. S. C. St. 1712, ch. 317; *infra*, p. 123.

<sup>92</sup> Mass. St. 1701-2, ch. 9; 1849, ch. 211.

<sup>93</sup> N. Y. St. 1794, ch. 53.

<sup>94</sup> N. Y. St. 1832, ch. 333; 1850, ch. 324.

<sup>95</sup> N. Y. St. 1796, ch. 38; 1805, ch. 31; 1897, ch. 378, sec. 1221.

<sup>96</sup> N. Y. St. 1823, ch. 71.

<sup>97</sup> N. Y. St. 1811, ch. 175; 1897, ch. 378, secs. 1219, 1220; cf. S. C. St. 1760, ch. 892; *infra*, p. 124. In Louisiana the police juries of the respective parishes have had control of local quarantine since 1835. (1835, ch. 162).

<sup>98</sup> N. Y. St. 1893, ch. 661.

<sup>99</sup> Mass. St. 1879, ch. 201; *Columbia Univ. Studies*, 8, p. 64; 9, pp. 124-47. See also S. C. St. 1878, ch. 610; 1901, ch. 420; Fla. R. S. 1892, tit. XI; La. St. 1898, ch. 192.

of the last century has been more and more toward a statement of broad principles, leaving much to the discretion of a health officer with ample power to make orders and give directions in individual cases. In Massachusetts, on the contrary, few details have ever been given in the laws; everything has been left to the regulations and orders made by the local administrative authorities.

2. *Nuisances*.—Control over local matters relating to health is probably inherent in local authorities, although there is a difference of opinion on the subject.<sup>100</sup> If, in the face of great danger to the public health, the regularly constituted authorities fail to act, it is quite certain that the inhabitants will take matters into their own hands, as in Philadelphia during the yellow fever epidemic in 1793.<sup>101</sup> "Government of almost every kind was almost wholly vacated, and seemed by universal consent to be vested in the committee" of citizens appointed at a mass meeting called by the mayor. So also in St. Louis, where, during a bad epidemic of cholera in 1849, the mayor, common council, and health officers were deposed by a mass meeting of citizens, ward committees of public health appointed with absolute power, and disobedience of their orders punished by fine and imprisonment.<sup>102</sup>

Local health matters, aside from quarantine, fall generally under the head of nuisances, caused by noxious trades, or by filth of various kinds. Just how close a relation the nuisances caused by noxious trades bear to public health it is not necessary to discuss here. It is sufficient that the relation has been supposed to be an important one and that the control over such trades has been based largely upon the power to preserve the public health.

As far back as 1856, when New York was still under Dutch rule, the city records show an ordinance regulating the slaughter of cattle, and under the first English governor in 1665 a license or "ticket of consent" was required from the "agent of the corporation" before a butcher could prosecute his trade.<sup>103</sup> In 1676 there was an ordinance granting a franchise for a public slaughter-house to be built for the use of the city and forbidding the slaughter of cattle in other places.<sup>104</sup>

<sup>100</sup> 28 Cyc., p. 709.

<sup>102</sup> Wendt, *Asiatic Cholera*.

<sup>101</sup> See Account of Mathew Carey.

<sup>103</sup> Cooper v. Schultz, 32 How. Pr. 107.

<sup>104</sup> *Min. Com. Council*, 1, pp. 46, 67; 5, pp. 226, 302, 322, 357, etc.

Attempts were also made in those early days to regulate the nuisances arising from filth. In spite of proverbial Dutch cleanliness, the inhabitants of New Amsterdam seem to have managed their municipal housekeeping in a most unsanitary way. After the English occupation in 1668 we hear of Governor Lovelace complaining that he "could not get an obstinate and wilfully negligent people to pave the streets, or keep them or the wharves and dikes clean from filth and garbage," and within three months of his coming a severe epidemic, possibly yellow fever, visited the city. Whereupon was proclaimed a day of humiliation and prayer.<sup>105</sup> In 1647 the city council passed an ordinance, frequently revived, requiring householders to cleanse the streets, "every person cleansing before his or her dores," but upon each renewal of the ordinance complaints were registered of lack of observance, and in 1685 constables were required to see that former orders as to street cleaning were observed.<sup>106</sup> The inherent authority of municipalities to regulate such matters when they arise upon the public streets is probably upon a firmer basis than the regulation of noxious trades, since the streets are public property.

Up to this time there had been no statutory authority to control nuisances conferred upon the city government, and although the Dongan charter in 1686 might be considered authority for later ordinances its terms were vague and indefinite. Under this charter, and similarly under the later Montgomerie charter in 1730, general powers were granted the city council "to make laws, orders, ordinances, and constitutions . . . for the good rule, oversight, correction, and government of said city . . . and for the several tradesmen victuallers, artificers, and all other people and inhabitants." Ordinances were to endure only three months (under the Montgomerie charter twelve), unless allowed and confirmed by the governor. Acting under these general powers the municipal council passed a series of ordinances relating to nuisances, of which the execution was placed in various hands. In 1695 a supervisor of street cleaning was appointed; in 1699 the mayor was authorized to appoint public scavengers; in 1702, "the year of the great sickness," constables and sheriffs were ordered to carry out the ordinances for cleanliness,

<sup>105</sup> *Mem. Hist. N. Y.*, I, p. 346.

<sup>106</sup> *Min. Com. Coun.*, I, pp. 7, 13, 28, 167.



and in the following year, 1703, alderman were required to make presentment of all persons who neglected or refused to clean their streets.<sup>107</sup>

When the matter of nuisances first became a subject of legislative action in New York, it was still uncertain in the minds of the legislators who would be the proper authorities to administer these laws. Sometimes, therefore, the control over nuisances was vested by statute in the quarantine authorities, appointed by the governor;<sup>108</sup> sometimes in the regular municipal authorities, either alone or in connection with the state-appointed authorities.<sup>109</sup> It is to be noted in cases of mixed administration found in early New York laws that there was less opportunity for a conflict of authority than appears on the surface, since the mayor was appointed by the governor until 1821, and the whole administration of health matters was therefore ultimately under the control of the central authority up to that time. The statutes of those early days are not always perfectly clear as to the persons who should actually execute the laws, ordinances, and orders with regard to nuisances, although in general the commissioners of health were the executive agents. In Massachusetts the idea of local self-government was strongly intrenched from the beginning. Towns doubtless considered themselves empowered to control nuisances under the general grant of 1635<sup>110</sup> but it is difficult at this day to find definite records of such actions. After 1692<sup>111</sup> the power was granted by statute to selectmen of towns to assign places for slaughter-houses, although after 1710 in cases of nuisance arising from slaughter-houses there was provision for a jury of inquiry.<sup>112</sup>

Administration of health laws regarding nuisances by boards especially appointed for that purpose was the next stage of development. In New York City a board of health with power to control nuisances is first mentioned in the law of 1805.<sup>113</sup> The board was to

<sup>107</sup> *Min. Com. Coun.*, 1, p. 376; 2, pp. 95, 195, 246.

<sup>108</sup> N. Y. St. 1797, ch. 16; 1796, ch. 65; 1801, ch. 92; cf. Philadelphia, Pa. St. 17 Mar. 1806; 31 Mar. 1812; New Orleans, La. St. 17 Mar. 1818.

<sup>109</sup> N. Y. St. 1796, ch. 38; 1799, ch. 70; 1805, ch. 31.

<sup>110</sup> Rec. of Colony of Mass. Bay, 1, p. 172, Mar. 3, 1635; *supra*, p. 51.

<sup>111</sup> Mass. St. 1692, ch. 23; *supra*, p. 51.

<sup>112</sup> *Infra*, p. 90; cf. parish administration of health laws under La. St. 1835, p. 162.

<sup>113</sup> N. Y. St. 1805, ch. 31; also 1811, ch. 175; 1820, ch. 229. Similar mixed boards are found in La. St. 17 Feb. 1821, for New Orleans; 2 May 1874, for Shreveport; and 20 Mar. 1878, for Baton Rouge; see also Pa. St. 1850, ch. 395; *supra*, p. 13.



consist of the commissioners of the health office appointed by the governor and such other members as the mayor and council might appoint. In 1823<sup>114</sup> the commissioners were no longer members of the board, but were still the executive officers, the board consisting solely of appointees of the city council. In 1849<sup>115</sup> a new executive department, that of the city inspector, was created, and the following year<sup>116</sup> the city inspector became the executive officer of the board, now the mayor and council; the commissioners, aside from the duties imposed by statute of visiting the sick and performing other professional duties as required, were to advise the board of health. Except for the interim from 1850-70, during the first part of which the mayor and council administered the health laws as a board of health, and during the latter part of which the Metropolitan Board of Health performed those duties, there has been a board of health in charge of the health interests of New York City from 1805 to the present time. Even the Metropolitan Board, from 1866-70, was the city board of health in all but name, and exercised exclusive jurisdiction in health matters throughout the city and the Metropolitan Health District.<sup>117</sup> Since 1866 the board has been composed of working members who are paid salaries, and who take a professional rather than a political interest in the administration of the laws. Yet in this connection it is well to bear in mind that the health officer of the port, a state official<sup>118</sup> appointed by the governor, has been *ex officio* a member of the city board of health except from 1823-66, and even during these years was one of the commissioners, the executive agents of the board. Subordinates for the execution of the laws have been appointed since 1866 by the board. Chief among these subordinates has been the sanitary superintendent with general supervision over the execution of the orders of the board, with assistants and sanitary inspectors as needed. During the greater part of this time there has been also provision for a detail of police at the request of the board.<sup>119</sup>

Outside of New York City boards of health do not appear in the state until 1832, the year of the first great cholera panic.<sup>120</sup> Indeed it is somewhat difficult to discover just how local health

<sup>114</sup> N. Y. St. 1823, ch. 71.

<sup>118</sup> *Young v. Flower*, 22 N. Y. Supp. 332.

<sup>115</sup> N. Y. St. 1849, ch. 187.

<sup>119</sup> N. Y. St. 1882, ch. 410, ch. 3, sec. 296.

<sup>116</sup> N. Y. St. 1850, ch. 275; *supra*, p. 15.

<sup>120</sup> N. Y. St. 1832, ch. 333; *supra*, pp. 17, 23.

<sup>117</sup> *Jamaica v. Long Isd. R. R. Co.*, 37 How. Pr. 379.

affairs were administered in smaller places before this time. Local quarantine had been enforced by two justices of the peace since 1794, and city and village charters often gave powers ample enough to include the suppression of nuisances, but whether in other places nuisances were suppressed by justices of the peace or by county officers, or whether the New York towns were in the happy condition of having no nuisances to suppress, it has been impossible to discover. The law of 1832 was a temporary measure passed in an emergency; a permanent law providing for local boards of health in all cities, villages, and towns was not passed until 1850.<sup>121</sup> This law was a revival of that of 1832, and as both were passed under the pressure of a threatened epidemic of cholera, the quarantine features were more prominent than those relating to nuisances. Boards of health were to make and publish rules and regulations for the suppression and removal of nuisances, to appoint a health officer, prescribe his duties, and employ such other persons as were necessary to carry their rules and regulations into effect.

There has been no change since that time in the authorities who administer the law with regard to nuisances except that the local health officer since 1895<sup>122</sup> has been by statute the chief executive officer of the board. Since 1903,<sup>123</sup> however, the administration in the last analysis is under state control since the local health officer is now appointed by the state commissioner upon nomination of the local board.

In Massachusetts administration by boards of health, while permitted for towns as far back as 1797<sup>124</sup> for nuisances other than noxious trades, was not very general. Except for the brief interval in which Boston, Salem, and a few other large towns enjoyed elective boards of health created by special statutes, there were few boards chosen. Upon the granting of city charters a step backward was taken, as no special boards of health were required in most of the charters; health matters were administered by the mayor and council acting through committees, as in Boston, or through the city marshal, as in Salem. In 1849<sup>125</sup> by a general law the same freedom of choice as to methods of administration was granted to city councils in all

<sup>121</sup> N. Y. St. 1850, ch. 324.

<sup>124</sup> Mass. St. 1797, ch. 16.

<sup>122</sup> N. Y. St. 1895, ch. 203.

<sup>125</sup> Mass. St. 1840, ch. 211; *supra*, p. 54

<sup>123</sup> N. Y. St. 1903, ch. 383; 1904, ch. 484.

cities together with the powers of town boards. In 1866<sup>126</sup> committees or boards of health in cities were authorized to appoint agents to act in time of emergency or when the board could not assemble, such agents having all the authority of the board. It was not until 1877 that distinct boards of health were required in cities voting to accept the law, and not until 1895 that they were compulsory for all cities except Boston.<sup>127</sup>

The administration of laws regarding nuisances arising from slaughter-houses has been divided since 1871<sup>128</sup> between state and local authorities by requiring licenses from the local health authorities for persons about to engage in the business of slaughtering, and by giving the state board power after notice and hearing to order the cessation of offensive trades. It is noteworthy that in Massachusetts, the home of local government, the state board from its inception should have taken a vital interest in this class of nuisances which in New York have been left exclusively to the control of localities. The powers of the state board over such nuisances and over sewage disposal<sup>129</sup> are executed by the secretary.

3. *Vaccination*.—In no field of activity on behalf of public health has there been more variety of administrative authority than in that of vaccination. The early Massachusetts laws relating to inoculation with smallpox<sup>130</sup> were restrictive rather than mandatory. The first statute<sup>131</sup> requiring general vaccination, irrespective of the prevalence or menace of an epidemic, placed the execution of the law in the hands of the town boards of health where such existed, and made it the duty of towns having no board of health to elect three superintendents of vaccination. The expenses in either case were to be met by taxation.<sup>132</sup> The Revised Statutes in 1836<sup>133</sup> altered the law, making it permissive for towns to require vaccination of all the inhabitants or not. In 1855<sup>134</sup> the requirement was again made general, with the administration of the law under the supervision of the local govern-

<sup>126</sup> Mass. St. 1866, ch. 271.

<sup>127</sup> Mass. St. 1877, ch. 133; 1895, ch. 332.

<sup>128</sup> Mass. St. 1871, ch. 167; 1901, ch. 134; *supra*, pp. 55, 58.

<sup>129</sup> Mass. St. 1901, ch. 104; 1906, ch. 158.

<sup>130</sup> Mass. St. 1764, ch. 12; 1776-77, chs. 5, 7; 1792, ch. 58.

<sup>131</sup> Mass. St. 1809, ch. 117.

<sup>132</sup> Such a tax, without a special authorizing law, was upheld in Vermont, *Hazen v. Strong*, 2 Vt 427, 1830. See also an early N. H. case, *Wilkinson v. Albany*, 8 Foster 9, 1853.

<sup>133</sup> Mass. R. S. 1836, ch. 21.

<sup>134</sup> Mass. St. 1855, ch. 414.

mental authorities: selectmen of towns, the mayor and aldermen of cities; although the duty of excluding unvaccinated children from the schools was laid upon school committees. The administration of the law except as to schools was transferred to local boards of health in 1894<sup>135</sup> with discretionary power to require the revaccination of adults. The law was attacked as an unconstitutional violation of personal liberty but was upheld by the supreme courts both of the state, and of the United States.<sup>136</sup>

In New York the law of 1860<sup>137</sup> empowered school boards to exclude unvaccinated children from the schools. The public health act of 1893<sup>138</sup> went farther and forbade the admission of such children to the schools. The execution of both laws was intrusted to the school authorities. The constitutionality of the latter law was attacked<sup>139</sup> but the law was upheld on the ground that a common-school education is a privilege and not a right, that it is created by legislative enactment and subject to legislative regulation, and that the school officers have no discretion in the matter but must obey the law.

4. *Investigation*.—The possibility of investigating the causes of disease under state auspices was first included in a law in the New York cholera act of 1832,<sup>140</sup> by which the governor was empowered to employ agents to procure information as to the progress, prevention, and treatment of cholera; it was made his duty to communicate such information by proclamation.

The Massachusetts sanitary commission of 1850 recommended that investigations as to the causes of disease, especially consumption,

<sup>135</sup> Mass. St. 1894, ch. 515.

<sup>136</sup> *Com. v. Pear*, 183 Mass. 242, 1903; *Jacobson v. Mass.*, 197 U. S. 11.

<sup>137</sup> N. Y. St. 1860, ch. 438.

<sup>138</sup> N. Y. St. 1893, ch. 661, sec. 200.

<sup>139</sup> *In re Walters*, 32 N. Y. Supp. 322, 1895; *in re Viemeister*, 179 N. Y. 235, 1904.

NOTE.—In all cases in other states arising under statutes by which authority has been conferred upon local boards of health or school boards to require vaccination either before admission to the schools or otherwise the constitutionality of the statute has been upheld. Such cases are: *Abeel v. Clark*, 84 Cal. 226, 1890; *Bissell v. Davison*, 65 Conn. 183, 1894; *Morris v. Columbus*, 102 Ga. 792, 1898; *State v. Hay*, 126 N. C. 990, 1900. Where a statute has conferred no express power to require vaccination but the local authorities have required it under a general grant of power to promote the public health there has been more doubt in the minds of the courts. Where an emergency has existed they seem inclined to support the action of the local authorities: *Blue v. Beach*, 153 Ind. 121, 1900; *Duffield v. Williamsport School Dist.*, 162 Pa. St. 476, 1894; *Freeman v. Zimmerman*, 86 Minn. 353, 1902. Where no emergency has existed the trend of judicial opinion seems to be against a vaccination requirement: *Potts v. Breen*, 167 Ill. 67, 1897; *Lawbaugh v. Bd. Ed.*, 177 Ill. 572, 1899; *Adams v. Burdge*, 95 Wis. 390, 1897; *Mathews v. Bd. Ed.*, 127 Mich. 530, 1901; although the Missouri courts took the opposite view: *In re. Rebenek*, 62 Mo. App. 8, 1895.

<sup>140</sup> N. Y. St. 1832, ch. 333.



should be made by local boards. The idea of a state board, created for purposes of investigation, was first presented in the third National Quarantine and Sanitary Convention<sup>141</sup> held in New York City in 1859, but it was 20 years after the recommendation of the Massachusetts commission and 10 after that of the National Sanitary Convention before the first state board, that of Massachusetts, was established.

Meanwhile, in 1866,<sup>142</sup> it was made the duty of the Metropolitan Board in New York "to gather and preserve information and facts relating to deaths, disease, and health," as far as it was able without serious expense, and keep in communication with local boards.

The duties laid upon the New York and Massachusetts state boards by the statutes<sup>143</sup> establishing them were given in almost identical phrases: to take cognizance of the interests of life and health among the people of the state, to investigate the causes of disease, especially epidemics, the sources of mortality, the effects of localities, employments, and conditions upon public health, and to collect and preserve information. These duties have been performed by the members of the boards themselves, or by experts acting under their guidance and employ. Other duties, more strictly administrative, have been laid upon state boards by later statutes, but the importance of the scientific investigations conducted by them has steadily increased. Their annual reports are filled with the results of such investigations.

With the investigative work of the boards has been included the diffusion of sanitary knowledge, both for the education of local boards and health officers, and for the information of the people at large; a glance at the latest appropriation acts in the two states under consideration will show thousands of dollars set aside for such purposes as a traveling tuberculosis exhibit, the expenses of a conference of health officers, and for the publication and distribution of special pamphlets to inform the people as to the best means of preventing tuberculosis and other diseases.<sup>144</sup> To investigation one other sphere of activity has been added, scarcely governmental: the manu-

<sup>141</sup> *Proceedings and Debates*, Third Natl. Quar. and Sanit. Conv., p. 88.

<sup>142</sup> N. Y. 1866, ch. 74, sec. 18.

<sup>143</sup> Mass. St. 1869, ch. 420; N. Y. St. 1880, ch. 322.

<sup>144</sup> Mass. St. 1906, ch. 47; 1907, chs. 84, 208, 364, 537—total \$134,500; N. Y. St. 1907, chs. 577, 578,—total, aside from food inspection, \$128,170.



facture and distribution of pure vaccine virus and of antitoxins for diphtheria, tetanus, and other diseases. Allied to this non-governmental activity of the state is the power granted to cities of over 250,000 inhabitants in New York<sup>145</sup> to establish under certain conditions tuberculosis sanatoria outside of their own limits.

From the first health laws of two hundred years ago, limited in scope to the exclusion of infection by means of quarantine and isolation, it is a long road to the present highly organized departments of health, exercising ample powers for the investigation and prevention of disease in all its forms.

<sup>145</sup> N. Y. St. 1899, ch. 637.

## CHAPTER 4.

### ENFORCEMENT.

THERE are two general methods of enforcement of health laws, whether statutes, regulations, or orders. The first is through punishment after judicial decision, the second by specific enforcement; the latter may be carried out through the courts, or directly through the health authorities.

#### A. ENFORCEMENT BY PUNISHMENT.

Punishment may be in the nature of fine or imprisonment after conviction of a misdemeanor; or a penalty may be recovered on suit.

Early English statutes made offenses against the quarantine laws a felony, and by the common law the maintenance of a public nuisance was a misdemeanor, and hence an indictable offense; but for a private nuisance, since it was not a misdemeanor, the remedy was by action on the case.<sup>1</sup> In this country the only law, as far as ascertained, by which offenses against quarantine were made a felony was an act of the Massachusetts Bay Colony of 1721-22<sup>2</sup> by which the master or captain of any vessel refusing to perform the quarantine required in the law "shall be deemed a felon, and shall suffer the pains of death." But this law was an exceptional one, passed through fear of the plague, and was limited to three years. Indeed, even criminal actions were not always provided in the health laws, offenses against them were not always made misdemeanors, although it must be borne in mind that the distinction was not clearly held in the early laws between conviction of misdemeanor with payment of a fine, and a suit for penalties. In Massachusetts the early quarantine laws were generally enforced by penalties, although as there has never been a general quarantine law for the state, the penalties are imposed for violation of regulations of town boards of health. In New York, on the other hand, while penalties were sued for before

<sup>1</sup> Blackstone, IV, pp. 161, 167; Broom's *Common Law*, p. 821.

<sup>2</sup> Mass. Prov. Laws 1721-2, ch. 3; the earliest quarantine law (Pa. 1700) provided for a penalty of £100 to be recovered for the use of the proprietary and governor of the province.

1796, since that date offenses against quarantine have been uniformly treated as misdemeanors.<sup>3</sup>

The same contrasting tendencies are to be noted between New York and Massachusetts legislation with regard to punishment for nuisances, although they are not so clearly marked as in regard to quarantine. In New York with few exceptions violations of statutory provisions, regulations of boards of health, or ordinances of city authorities have been considered misdemeanors; non-compliance with the orders of the Board of Health of New York City has also been made a misdemeanor in many statutes from the very beginning of nuisance legislation.<sup>4</sup> The act of 1850<sup>5</sup> establishing local boards gave them power to make regulations concerning nuisances and made a violation of such regulations a misdemeanor, but made no mention of orders. In a case coming before the courts in 1854 it was held that this provision did not apply to *orders* of the board of health.<sup>6</sup> The law of 1867 for local boards remedied the defect, and violations of orders of boards of health as well as of regulations concerning nuisances have since that time been misdemeanors.<sup>7</sup> Few instances of penalties occurred in the New York City health laws until the establishment of the Metropolitan Board of Health in 1866. By that act, in addition to punishment for a misdemeanor, a penalty up to \$250 might be sued for by the board; this provision has been continued in later laws relating to New York City and since 1870 has also occurred in laws for local boards.<sup>8</sup> By the law of 1885<sup>9</sup> regulations of the state board of health as to preserving water courses from contamination might be enforced by either civil or criminal process, but not by both.

But while enforcement by punishment as for misdemeanors is the rule, and suit for penalties the exception in New York health laws regarding nuisances, in Massachusetts both methods have been employed from early days with the tendency toward suit for penalties. The law of 1785<sup>10</sup> as to noxious trades, indeed, provides for three

<sup>3</sup> N. Y. St. 1796, ch. 38.

<sup>4</sup> N. Y. St. 1798, ch. 65; 1823, ch. 71; 1850, ch. 275; 1866, ch. 74, Met. Bd.; 1867, ch. 956, sec. 17, etc.

<sup>5</sup> N. Y. St. 1850, ch. 324.

<sup>6</sup> Reed v. P., 1 Park Cr. 481, 1854; see also H. Dept. v. Knoll, 70 N. Y. 530, 1877.

<sup>7</sup> N. Y. St. 1867, ch. 790; 1905, ch. 443.

<sup>9</sup> N. Y. St. 1885, ch. 543.

<sup>8</sup> N. Y. St. 1866, ch. 74, sec. 30; 1870, ch. 559.

<sup>10</sup> Mass. St. 1785, ch. 1: *infra*, p. 90.

methods of enforcement: by fine, after conviction on presentment to the Grand Jury; by suit for penalty; and by suppression after an inquiry by a jury. The various revisions of the statutes have preserved both the method of abatement of a nuisance after inquiry by a jury, and the imposition of penalties on suit, although there seems to be some discrepancy between the earlier law and the Revised Statutes, as to the inquiry by jury.<sup>11</sup> Also it has been held that statutory provisions have not repealed the remedy of indictment at common law.<sup>12</sup> The reoccupation of unfit dwellings after eviction by the board of health was made an indictable offense in 1850,<sup>13</sup> and neglect of orders of the board as to noxious trades in cities which had voted to accept the law in 1855.<sup>14</sup> Penalties were imposed, to be recovered by suit, for offensive trades;<sup>15</sup> for failure to obey orders of the board of health or health officers as to nuisances,<sup>16</sup> and for resisting search for nuisances in Boston.<sup>17</sup>

*Qui tam actions.*—In early times the duty of enforcing the health laws by information or complaint was not laid upon any special officer and we therefore find frequent instances of an inducement held out to any person who would enter suit or make complaint.<sup>18</sup> In Massachusetts in 1718<sup>19</sup> violations of quarantine were punishable by fines to be sued for by the province treasurer, one-third to go to His Majesty's government, one-third to the informer, and one-third to the province treasurer for the time being. A similar provision, one-half to the person bringing suit, is found in the laws of 1785 and 1799<sup>20</sup> for abating nuisances caused by noxious trades. From the time the Revised Statutes were passed in 1836 such inducements were no longer offered to those bringing offenders against the health laws to justice.

<sup>11</sup> Mass. R. S., ch. 21, sec. 12; G. S., ch. 26, sec. 12; P. S., ch. 80, sec. 25; R. L., ch. 75, sec. 72, as to jury; R. S., ch. 21, sec. 5; G. S., ch. 26, sec. 5; P. S., ch. 80, sec. 18; R. L., ch. 75, sec. 65, as to penalties.

<sup>12</sup> *Com. v. Rumford Chem. Works*, 16 Gray 231, 1860.

<sup>13</sup> Mass. St. 1850, ch. 108.

<sup>16</sup> Mass. St. 1797, ch. 16.

<sup>14</sup> Mass. St. 1855, ch. 391; *supra*, p. 54.

<sup>17</sup> Mass. St. 1799, ch. 10, and later statutes.

<sup>15</sup> Mass. St. 1799, ch. 75.

<sup>18</sup> Sometimes the inducements offered were very large, as in S. C. St. 1744, ch. 720, by which one-half of a cargo of negroes landed contrary to the law was to be given to any person bringing suit, and in S. C. St. 1738, ch. 651, by which half of the penalty of £500 imposed for inoculating with smallpox could be recovered by the one who should sue. See also S. C. St. 1712, ch. 317; 1783, ch. 1194; and Ga. St. 1793, 17 December; *infra*, p. 123.

<sup>19</sup> Mass. St. 1717-18, ch. 14; also 1739, ch. 1, and 1797, ch. 16.

<sup>20</sup> Mass. St. 1785, ch. 1; 1799, ch. 75.

In New York the first quarantine law provided that one-third the penalty of £500 should go to the person who sued for it, but in 1784 it was made the duty of the warden of the port to bring suit.<sup>21</sup> In 1797<sup>22</sup> one-half the penalty of \$100 for carrying on an offensive trade in the part of the city where such trades were forbidden by statute was given to the person who sued, but this statute was of short duration. The only recent approach to *qui tam* in New York is in the law of 1870<sup>23</sup> forbidding the deposit of refuse on or near highways or navigable streams, by which any person aggrieved might serve notice upon the offender for the removal of the nuisance with the right to sue for \$25.00 for each day of neglect to remove.

#### B. SPECIFIC ENFORCEMENT.

In both of these methods of enforcement by punishment—after conviction as a misdemeanor and suit to recover a penalty—the accused was given the safeguard of the processes of the law, but these processes were slow and often the very essence of authority to deal with matters relating to health must lie in the speedy enforcement of the law. Long before punishment can be meted out for a broken law irreparable damage may be done. The balance is a nice one, at once to protect the personal and property rights of the individual and not to neglect the demands of the public welfare. In the preamble of an early Massachusetts act<sup>24</sup> we find the legislature expressing the necessity for more speedy and more efficient enforcement of the laws: "Whereas the laws now in force are inadequate to so speedy a removal of nuisances as the Exigencies of the public may require. . . ." The demand for quicker enforcement, whether expressed in preambles or not, led to various methods of specific enforcement. Such enforcement of health laws might be had either: (a) through the courts; or (b) without recourse to the courts, by the action of the health authorities themselves, either in a quasi-judicial capacity or by summary proceeding. The advantage of enforcement through the courts lies in the greater protection to individual rights and in the freedom from personal liability of the persons (officers of the law) carrying out the decrees of enforcement. On the other hand enforcement directly by health authorities makes for celerity and efficiency.

<sup>21</sup> N. Y. St. 1755, ch. 973; 1784, ch. 57.

<sup>22</sup> N. Y. St. 1797, ch. 16.

<sup>23</sup> N. Y. St. 1870, ch. 525.

<sup>24</sup> Mass. St. 1801, ch. 16.



### 1. *Enforcement through the Courts (Judicial Enforcement).*

a) *By warrant.*—Perhaps the earliest form of enforcement of health laws through the courts was by warrant issued by a justice of the peace in Massachusetts to a sheriff or constable for the return to his vessel of a person coming on shore in violation of quarantine.<sup>25</sup> The same use of a warrant directed to the sheriff or constable is found for the removal of persons coming from infected places who refused to withdraw from a town on notice,<sup>26</sup> and for the detention and purification of suspected baggage;<sup>27</sup> this provision is still in force.<sup>28</sup> A warrant from a justice of the peace was also required for forcible entry to search for or abate a nuisance in Boston<sup>29</sup> and in towns generally.<sup>30</sup>

In New York a warrant was required in towns from a justice of the peace for the removal of smallpox patients by the temporary law of 1778;<sup>31</sup> and as the execution of the quarantine law for towns other than New York City was in the hands of two justices of the peace it is fair to suppose that the method of enforcement intended by the law was by means of warrants.<sup>32</sup> No warrant seems to have been required in New York City in early days for the removal of infected persons, but from 1798 to 1811<sup>33</sup> the board of health might require "the aid of a justice and constable" for forcible entry to examine for nuisances, and a warrant from the mayor or recorder, both having judicial powers, to the sheriff to abate. Where the statute does not distinctly provide for the method of removal of a nuisance the early decisions held that it might be abated by anyone acting under the common law, but such persons acted at their peril; the advantage of the warrant was, therefore, in the protection granted to those removing the nuisance.<sup>34</sup> Later court decisions have overthrown this doctrine and have held that a public nuisance might be removed only by a person especially injured thereby.

<sup>25</sup> Mass. St. 1701-2, ch. 9; by whom such a warrant was to be procured is not always clear; the law seems to impose administrative duties upon the justices of the peace for marine quarantine (sec. 4).

<sup>26</sup> Mass. St. 1739, ch. 1.

<sup>27</sup> Mass. St. 1751-2, ch. 12; 1797, ch. 16; R. S., ch. 21, secs. 19, 20.

<sup>28</sup> R. L., ch. 75, secs. 46, 87.

<sup>30</sup> Mass. R. S., ch. 21, sec. 18.

<sup>29</sup> Mass. St. 1799, ch. 10.

<sup>31</sup> N. Y. St. 1778, ch. 36.

<sup>32</sup> N. Y. St. 1794, ch. 53; 1801, ch. 92; R. S., ch. 14, tit. 6, sec. 22, by "any order in writing."

<sup>33</sup> N. Y. St. 1798, ch. 65; 1811, ch. 175.

<sup>34</sup> *Hart v. Mayor Albany*, 9 Wend. 571; *Van Wormer v. Albany*, 15 Wend. 262; *infra*, p. 108, "Liability."

b) *Inquiry by jury*.—Another form of enforcement, found in Massachusetts, is enforcement after inquiry by a jury. This is probably derived from the old English "hundred jury" in attendance upon the quarter sessions, whose business it was "to inquire of such things as shall be given them in charge," including nuisances.<sup>35</sup> Very similar were the inquiries which the Court of General Sessions of the Peace of the county, by the laws of 1710 and 1785, might cause to be made by a jury into nuisances caused by noxious trades,<sup>36</sup> followed by suppression of the nuisance, either by "prohibiting and restraining," or by "causing such nuisances to be removed or prevented." In 1801<sup>37</sup> this was called a "Court of Inquiry" and was summoned by two justices of the peace. That it was an administrative proceeding is clearly shown by the fact that there was no indictment, no punishment of an offender; the jury was summoned to determine the fact of the existence of a nuisance and to order its abatement and removal. No mention is made of such inquiry by a jury in the Revised Statutes, although they provided that whenever a building which had been assigned for a noxious trade became a nuisance, and it should be so made to appear before the Court of Common Pleas (which implied a jury), the court might revoke the assignment, prohibit the further use of the building for such purposes, and cause the nuisance to be removed.<sup>38</sup> This provision is still embodied in the laws.<sup>39</sup> The jury of inquiry reappears in the law of 1855<sup>40</sup> by which appeals from orders of the board of health are made to a jury impaneled for that purpose, with power to render a verdict affirming, annulling, or altering the order of the board from which appeal was taken. There is no inquiry of this kind by a jury preceding abatement to be found among the health laws of New York.<sup>41</sup> Indeed in the case of *Met. Bd. v. Heister*<sup>42</sup> it was stated by the court,

<sup>35</sup> Webb, S. and B., *English Local Government*, p. 456.

<sup>38</sup> Mass. R. S., ch. 21, sec. 48.

<sup>36</sup> Mass. St. 1710-11 ch. 8; 1785, ch. 1.

<sup>39</sup> Mass. R. L., ch. 75, sec. 92.

<sup>37</sup> Mass. St. 1801, ch. 16.

<sup>40</sup> Mass. St. 1855, ch. 301.

<sup>41</sup> The only jury summoned in connection with the removal of noxious trades in New York was that provided for by the laws of 1796, ch. 38, and 1797, ch. 16, by which a jury was to fix the compensation to be paid to owners of noxious establishments ordered removed for the loss occasioned by the removal and then only in case an agreement could not be reached between the mayor and owner as to the amount of compensation to be paid to the owner. Perhaps it should be noted here that in the law of 1797 after a jury had fixed the amount of compensation it was optional with the mayor and council whether to pay the amount fixed, or to make the removal to another location at the expense of the city.

<sup>42</sup> *Met. Bd. v. Heister*, 37 N. Y. 661, 1868; see also *Reynolds v. Schultz*, 34 How. Pr. 147, 1868.

after citing a series of early laws, that "the absolute control over persons and property, so far as public health was concerned, was vested in boards or officers who exercise a summary jurisdiction over the subject" and that a jury had not been the ordinary tribunal to determine questions of nuisances prior to the adoption of the constitution of 1846 and hence was not included in the constitutional provision saving jury trial in all cases in which it has been used before that time.

c) *Abatement by order of court after conviction*.—To be distinguished from this method of abatement of a nuisance after inquiry is abatement by order of the court after conviction of a misdemeanor; in the first instance the business of slaughtering or other noxious trade had become a nuisance (in 1710 the Massachusetts legislature said it might become such by the growth and increase of the town), although carried on in a place assigned by the local authorities or selectmen, and was therefore subject to inquiry; in the second, the trade was carried on, or the nuisance arose in contravention of some order<sup>43</sup> of the selectmen or board of health and hence was subject to punishment.

The power of the court to order abatement after conviction under the common law without express statutory grant is unquestioned. "By all the authorities the defendant may be required as part of his sentence to abate the nuisance;" neglect or refusal is considered contempt of court and punished as such.<sup>44</sup>

In Massachusetts, however, since 1836 abatement after conviction has been expressly provided in the statute.<sup>45</sup>

d) *Enforcement by injunction*.—Still another method of enforcement through the courts has been used—that by injunction. There has been some question whether a court of equity could enjoin a common nuisance without statutory authority, but in Massachusetts such enforcement was provided by statute as early as 1827.<sup>46</sup> Equity jurisdiction was then so new in the minds of the judges that in a case coming before them within a few months, while admitting the desirability of such restraints in regard to private nuisances, the court was

<sup>43</sup> Mass. St. 1785, ch. 1, in a place not assigned.

<sup>44</sup> Bishop, *New Criminal Procedure*, II, sec. 871, 1; *Clark v. Syracuse*, 13 Barb. 32, p. 37.

<sup>45</sup> Mass. R. S., ch. 21, sec. 12; R. L., ch. 75, sec. 72.

<sup>46</sup> Mass. St. 1827, ch. 88.

loath to express an opinion as to the applicability of the statute to public nuisances.<sup>47</sup> But the intent of the legislature, if doubtful before, was made quite clear in the Revised Statutes,<sup>48</sup> and the right to apply for injunction to enforce orders for the removal of nuisances has belonged to towns and cities in Massachusetts by statute and court decision from that time to this.<sup>49</sup> Indeed the law of 1855<sup>50</sup> went farther and provided in the statute itself for the suspension of a noxious trade after an order by a board of health to suspend and pending an appeal for a jury. The court upheld this provision as being<sup>51</sup> upon the same ground as an injunction, i. e., the prompt suppression of evils. Indeed in later cases<sup>52</sup> we hear of the general jurisdiction of a court of equity over nuisances without special statutory authority, and that a statute authorizing a board to "take all necessary measures to prevent" gives power without other special authority to bring suit in equity in the name of a town to suppress a nuisance. This is a far road from the doubtful assent of the court in 1828 to equity jurisdiction in any case of nuisance.<sup>53</sup>

In New York the legislature was much later in making direct statutory provision for restraint by injunction,<sup>54</sup> although as far back as 1842 the court had granted an injunction to prevent a private nuisance arising from a slaughter-house.<sup>55</sup>

That a court of equity could always interfere by injunction and without a jury to restrain the continuance of a business so conducted that it was a nuisance, and that the power was not only conferred by statute, but belongs to the general powers possessed by courts in equity, has been held in several New York cases,<sup>56</sup> and even that the power

<sup>47</sup> *Chas. River Bridge v. Warren Bridge*, 6 Pick. 376, 1828.

<sup>48</sup> Mass. R. S., ch. 21, sec. 13.

<sup>49</sup> *Winthrop v. Farrar*, 11 Allen 398, 1865; *Watertown v. Mayo*, 109 Mass. 315, 1872; *R. L.* 1902, ch. 75, sec. 73.

<sup>50</sup> Mass. St. 1855, ch. 391.

<sup>51</sup> *Belcher v. Farrar*, 8 Allen 325, 1864.

<sup>52</sup> *Winthrop v. Farrar*; *Taunton v. Taylor*, 116 Mass. 254, 1874.

<sup>53</sup> See also Mass. St. 1871, ch. 167; 1880, ch. 193; 1893, ch. 460, for special statutory provisions as to injunction. But notice of the order must be given and the order must be reasonable, or the injunction will be denied; *Belmont v. New Eng. Brick Co.*, 190 Mass. 442, 1906.

<sup>54</sup> N. Y. St. 1867, ch. 956, for New York City; 1870, ch. 559, for local boards.

<sup>55</sup> *Catlin v. Valentine*, 9 Paige 575, 1842; *Brady v. Weeks*, 3 Barb. 157, 1848.

<sup>56</sup> *Reynolds v. Schultz*, 4 Rob. 282, 1867; *Dubois v. Budlong*, 15 Abb. Pr. 445, 1863; *H. Dept. v. Purdon*, 99 N. Y. 237, 1885; *Gould v. Rochester*, 105 N. Y. 46, 1887; *Bell v. Rochester*, 11 N. Y. Supp. 305, 1890; *Yonkers v. Copcutt*, 140 N. Y. 12, 1893.



conferred by the statute of 1867 extended beyond matters which were common nuisances to violations of orders of boards of health as to the manner and place in which they were to be carried out.<sup>57</sup>

2. *Specific Enforcement by the Health Authorities.*

Specific enforcement of health laws, regulations, and orders of boards of health may also be accomplished by the direct action of the health authorities. They may act in a quasi-judicial capacity or in a summary manner.

a) *By warrant (quasi-judicial).*—Attention has already been called to the enforcement of health laws by means of a warrant issued by a justice of the peace; very similar is enforcement under a quasi-judicial power granted to boards of health to issue warrants. The temporary cholera act of 1832<sup>58</sup> gave to local boards of health the power to issue warrants to a constable for the removal of infected persons, and if necessary, to the sheriff to bring to their aid the entire force of the county. The same power to issue warrants for the removal of infected persons reappears in the law of 1850 for local boards;<sup>59</sup> and in the law of the same year for the city of New York power is given to the board of health to issue warrants for the apprehension and removal of such persons "as cannot otherwise be subjected to the regulations by them adopted."<sup>60</sup> By the same law the health officer might "direct in writing" the constable or some other person to arrest persons escaping from quarantine. Until 1866 the issuing of warrants by the board of health was confined to cases of contagious diseases. In that year the Metropolitan Board of Health was given authority to arrest under its own warrant any person violating or resisting any law, ordinance, or order of the board. This provision was attacked as being contrary to the constitutional requirement of due process, but was upheld by the lower courts upon the ground that it was in reality only the power to bring before a magistrate.<sup>61</sup> It does not seem to have been brought before the Court of Appeals.

<sup>57</sup> H. Dept. v. Lator, 38 Hun 542, 1886; P. v. Rosenberg, 138 N. Y. 410, 1893; Cronin v. P., 82 N. Y. 314, 1880.

<sup>58</sup> N. Y. St. 1832, ch. 333.

<sup>59</sup> N. Y. St. 1850, ch. 324, sec. 7.

<sup>60</sup> N. Y. St. 1850, ch. 275, tit. 3, art. 1, secs. 5, 6.

<sup>61</sup> Cooper v. Schultz, 32 How. Pr. 107, 1866; Reynolds v. Schultz, 34 How. Pr. 147, 1867.



There seems to be no similar power to issue warrants given to boards of health in Massachusetts, although in Louisiana and other states following New York precedents in legislation a similar power is found.<sup>62</sup> The main advantage in a statutory grant of power to boards of health to issue warrants lies in the protection thus afforded to those carrying out the behests of the board. In New York where boards may issue warrants it is probable that officers are adequately protected.<sup>63</sup> On the contrary, where boards have no authority to issue warrants, while those carrying out their orders are protected for the time being because acting under orders of the board of health, yet "they act at their peril if it turns out in subsequent proceedings that there was in fact and in law no nuisance."<sup>64</sup>

b) *Enforcement of special orders by boards of health after notice and hearing (quasi-judicial).*—Notice and hearing, another quasi-judicial power of boards of health, does not appear in New York laws as essential to the enforcement of orders of a board of health in regard to nuisances until 1850. In that year the law for New York City<sup>65</sup> required the city inspector, upon complaint being made of any trade as a nuisance and detrimental to health, to give notice to the persons concerned to show cause before the board of health why such trade should not be discontinued. The order of the board of health given after a hearing was final and conclusive, and disobedience to such orders was made a misdemeanor. When the law was brought before the courts for interpretation they held that a resolution of the board of health directing a nuisance to be abated was void without such previous notice and hearing.<sup>66</sup> In cases arising under the law of the same year, 1850, applying to the state at large, which made no mention of notice and hearing, it was held<sup>67</sup> that the power to make regulations for the removal of nuisances did not include the power to make orders, on the ground that "it is impossible to think the legislature intended to confer on boards of health power to make an adjudication against an individual without notice and in his absence . . . ." involving pen-

<sup>62</sup> La. St. 1855, No. 336.

<sup>63</sup> *Ersine v. Hohnback*, 14 Wall. 613; *Chegaray v. Jenkins*, 5 N. Y. 376, p. 381, 1851.

<sup>64</sup> *Stone v. Heath*, 179 Mass. 385, 1901; *Miller v. Horton*, 152 Mass. 540, 1891.

<sup>65</sup> N. Y. St. 1850, ch. 275, tit. 3, art. 1, secs. 1, 3.

<sup>66</sup> P. v. Bd. H. N. Y. City, 33 Barb. 344, 1861.

<sup>67</sup> *Reed v. P.*, 1 Park Cr. 481, 1854; *Rogers v. Barker*, 31 Barb. 447, 1860.

alties, and that "the legislature never designed to commit power to a board of health to conclude a thing was a nuisance and order its destruction without opportunity to be heard." Nor were the powers of city councils acting under city charters interpreted more broadly. Under the charter of Syracuse,<sup>68</sup> it was held that the city council had no right "without trial or notice to the party interested, to destroy large and valuable property, under pretence that it is a nuisance endangering the health of the city," and that an injunction to restrain the board would be given. None of these cases reached the Court of Appeals, but the lower courts were quite consistently of the same opinion: that notice and hearing were necessary to the enforcement of orders.

The laws of 1866-67 establishing the Metropolitan Board of Health brought the whole subject of quasi-judicial powers prominently before the courts;<sup>69</sup> sec. 14 of the law of 1866, especially, was attacked as being unconstitutional. In the course of the decisions rendered the courts declared that the quasi-judicial functions conferred upon the board to issue warrants, give notice and hearing, compel witnesses<sup>70</sup> did not constitute a court and that redress from its actions could always be had in the regular tribunals.<sup>71</sup> In the only case among those brought against the Metropolitan Board in which the question of hearing was definitely brought up,<sup>72</sup> it was held that upon refusal to fix a day for a hearing of the party affected

<sup>68</sup> Charter of Syracuse, N. Y. St. 1847, ch. 652; *Clark v. Syracuse*, 13 Barb. 32, 1852.

<sup>69</sup> N. Y. St. 1866, ch. 74, 1866, ch. 686; 1867, ch. 700; 1867, ch. 956. In the cases brought against the Metropolitan Board of Health the attack was made on three grounds: (1) that the law delegated legislative power to an appointed board; (2) that it delegated judicial authority; (3) that the summary powers granted to abate nuisances were contrary to the constitutional requirements of "due process" and trial by jury. In upholding the constitutionality of the law the courts held with regard to these objections: (1) that the legislature could create new sanitary districts with appointive officers (*Met. Bd. v. Heister*, 37 N. Y. 661); (2) that the power to make regulations was not true legislation, such regulations being in the nature of administrative by-laws (*Cooper v. Schultz*, 32 How. Pr. 107; *Coe v. Schultz*, 47 Barb. 64), but for the board to declare a thing a nuisance which was not such at common law was legislative and *ultra vires* (*Mayor v. Bd. of H.*, 31 How. Pr. 385; *Schuster v. Met. Bd.*, 49 Barb. 450); it is to be noted in this connection that while the regulations of boards of health are not strictly legislation they may become such by adoption, the law may be built up in great part by such regulations; (3) that while it was within the province of the legislature to establish new courts and fix their jurisdiction, the quasi-judicial powers conferred on the board did not constitute it a court (*Met. Bd. v. Heister*; *Cooper v. Schultz*); (4) that abatement by the board was not a taking of property without due process (*Cooper v. Schultz*; *Weil v. Schultz*; *Coe v. Schultz*), and was less objectionable than abatement by private persons (*Coe v. Schultz*); (5) that a jury was not customary or necessary for determining the fact of a nuisance (*Reynolds v. Schultz*; *Met. Bd. v. Heister*).

<sup>70</sup> N. Y. St. 1867, ch. 956.

<sup>71</sup> *Cooper v. Schultz*, 32 How. Pr. 107; *Coe v. Schultz*, 47 Barb. 64; *Reynolds v. Schultz*, 34 How. Pr. 147; *Met. Bd. v. Heister*, 37 N. Y. 661; see also *Golden v. H. Dept.*, 21 App. Div. 420.

<sup>72</sup> *Reynolds v. Schultz*.

a mandamus would lie, and that it was a matter of grave doubt whether the legislature could "constitutionally authorize any person or body . . . to destroy property . . . without providing for a hearing before condemnation, or compensation."

Such notice and hearing were not explicitly required in the later general laws for local boards, although the power to make orders for the abatement of nuisances was given as well as other judicial powers, i. e., to issue warrants and subpoenas, compel witnesses, administer oaths with the same powers as justices of the peace in civil actions, and prescribe and impose penalties for violations of or failure to comply with orders or regulations<sup>73</sup> but for many years the courts held that such notice was essential and was implied in the statute:<sup>74</sup> "The statute does not in words require notice, but this is clearly implied. . . . The accused must be enabled to defend himself before final judgment."

But although the necessity for notice and hearing had been the steady doctrine of the lower courts, when the matter came before the Court of Appeals after the cholera scare of 1892<sup>75</sup> the opposite view was taken: that notice and hearing by the board of health were neither implied nor essential; that there could be no final determination as to the fact of the nuisance except by a regular court, nor without the appearance of the parties in such a court. "A hearing was not necessary, because the question of nuisance or not lies at the foundation of the jurisdiction, and the party proceeded against may always try that vital and decisive question in the courts and is not foreclosed by the order made." The same doctrine was laid down again in the famous case of *N. Y. H. Dept. v. Trinity Church* and more emphatically in the later case of *Cartwright v. Cohoes*.<sup>76</sup> "The board was not obliged to hear anybody. It could have acted upon its own inspection and knowledge of the premises." But hand in hand with the advancement of the doctrine that notice and hearing by a board of health were not necessary nor implied in the law went the other

<sup>73</sup> N. Y. St. 1893, ch. 661, sec. 21.

<sup>74</sup> *P. v. Bd. H. Seneca Falls*, 58 Hun 595, 1891; *P. v. Wood*, 62 Hun 131.

<sup>75</sup> *P. v. Bd. H. Yonkers*, 140 N. Y. 1, 1893; "when pestilence is forcing a way into our harbors and danger and death approach through all rot and filth, it is the condition with which boards of health must grapple and the condition which must be abated and removed without regard to the question who caused the trouble" (*Bd. H. v. Copcutt*, 140 N. Y. 12, 1893).

<sup>76</sup> *N. Y. H. Dept. v. Trinity Church*, 145 N. Y. 32, 1895; *Cartwright v. Cohoes*, 165 N. Y. 631, 1901.

doctrine that the question of the fact of the existence of the nuisance was always subject to investigation in court and that boards of health or health officers acted at their peril in abating a nuisance without the sanction of a court decision.<sup>77</sup> Where, however, even at the present time, local charters require notice and hearing by the board before the abatement of nuisances, lack of such notice will be held to invalidate the action of the board.<sup>78</sup>

In Massachusetts there was no trace of judicial power in the hands of health authorities until 1868. Indeed, as we have seen, the tendency was rather toward putting administrative functions in regard to health matters into the hands of the courts. By the law of 1868<sup>79</sup> boards of health of cities or towns were given power upon petition of those injured to abate nuisances caused by wet lands after notice and hearing. Under this law, if the board of health failed to take action, the petitioner might appeal to the Superior Court or to a justice thereof who might appoint commissioners to determine the question of nuisance after a hearing. Aside from the abatement of nuisances caused by swamp lands, the only other instance in Massachusetts in which the law requires notice and hearing by the board of health occurs in the law of 1871<sup>80</sup> by which the state board of health is given power to order the cessation of offensive trades in buildings where it is injurious to health in cities and towns of over 4,000 inhabitants, but such orders can be given only after notice and hearing. Actions by boards of health, either state or local, by virtue of power conferred by these acts, have been held invalid unless the required notice and hearing were given.<sup>81</sup> Lack of notice renders void assessments on the lands of owners not notified although the assessments are held legal on other lands involved. But if the law does not specifically require notice—and Massachusetts laws with these two exceptions do not—the serving of an order is sufficient guaranty of individual rights, as appeal from the order may be taken to a court;<sup>82</sup> and where no opportunity

<sup>77</sup> *Infra*, p. 108, "Liability."

<sup>78</sup> *Eckhardt v. Buffalo*, 19 App. Div. 1, 1897; also *Cushing v. Bd. H. Buffalo*, 13 N. Y. St. Rep. 783, 1887.

<sup>79</sup> Mass. St. 1868, ch. 160; *Grace v. Newton Bd. H.*, 135 Mass. 490, 1883.

<sup>80</sup> Mass. St. 1871, ch. 167.

<sup>81</sup> *Sawyer v. State Bd. H.*, 125 Mass. 182, 1877; *Watuppa Water Co. v. MacKenzie*, 132 Mass. 71, 1882; *Hall v. Staples*, 166 Mass. 399, 1896; *Belmont v. New Eng. Brick Co.*, 190 Mass. 442, 1906.

<sup>82</sup> *Belcher v. Farrar*, 8 Allen 325, 1864; *Com. v. Young*, 135 Mass. 526, 1893.



to be heard before a board of health is given, a party "is not concluded by the findings of adjudications of that board, and may contest all the facts upon which his liability is sought to be established."<sup>83</sup> And even where notice and hearing is given, the courts will read into the statute the opportunity for judicial review.<sup>84</sup> The final results, therefore, in Massachusetts and New York are not widely different, although reached by different roads; in both cases the final determination of the questions involved must be before the regularly constituted tribunals.

c) *Summary abatement, and enforcement of orders after notice, but without a hearing.*—The foregoing methods of specific enforcement, while more effective than punishment after conviction or suit for penalties, still left something to be desired in the way of speedy enforcement. Often the prompt suppression of evils could be obtained in no other way than by summary enforcement.

In regard to quarantine in particular, delay in enforcing the law might make its provisions of no avail. In Massachusetts as far back as 1701<sup>85</sup> we find selectmen of towns authorized to "make the best provision they can" for the preservation of the health of the inhabitants by the removal and isolation of infected persons, and in 1792<sup>86</sup> to remove, except at the risk of life. Vessels entering Boston harbor contrary to the orders of the board of health<sup>87</sup> might be forcibly removed to the quarantine ground.<sup>88</sup>

In New York persons leaving a vessel ordered to quarantine might be returned to the vessel by "force and violence,"<sup>89</sup> and after 1796<sup>90</sup> vessels lying at the wharves found to be in a condition prejudicial to public health might be forcibly removed to quarantine. The health officer of the port of New York has had power to destroy infected bedding or clothing since 1796;<sup>91</sup> in 1811<sup>92</sup> the commissioners of health were given power to destroy any cargo that was putrid "or in their

<sup>83</sup> *Salem v. East. R. R. Co.*, 98 Mass. 431, 1868.

<sup>84</sup> *Sawyer v. State Bd. H.*, 125 Mass. 182, 1877-78; *infra*, p. 105, "Remedies, Appeal."

<sup>85</sup> Mass. St. 1701-2, ch. 9.

<sup>86</sup> Mass. St. 1792, ch. 58.

<sup>87</sup> Mass. St. 1717-18, ch. 14; 1799, ch. 10, sec. 15.

<sup>88</sup> During the epidemic of yellow fever in 1819 when the vessel "Ten Brothers" arrived in so foul a condition that the custom house officer and ten laborers who boarded her died next night, the board of health went beyond forcible removal, for by their order the vessel was taken into the harbor and scuttled (*Mass. Sanit. Commis. Rep.* 1850).

<sup>89</sup> N. Y. St. 1755, ch. 973.

<sup>91</sup> N. Y. St. 1796, ch. 38.

<sup>90</sup> N. Y. St. 1796, ch. 38.

<sup>92</sup> N. Y. St. 1811, ch. 175.



opinion dangerous to the health of the city," and the power has been continued to the present time in substantially the same form.<sup>93</sup> By the law creating the Metropolitan Board of Health<sup>94</sup> persons infected with smallpox or other contagious disease might be removed and isolated at the discretion of the board. This power of summary removal of infected persons was upheld by the New York courts in a case<sup>95</sup> in which it was held that no action for damages would lie against an inspector who had removed a smallpox patient; but it must be shown that the person had been infected with or exposed to contagious or infectious disease as the power to isolate is dependent upon such infection or exposure. It has also been held that the board is *ultra vires* in quarantining a person who refuses to submit to vaccination.<sup>96</sup>

The necessity for speedy action for quarantine in times of emergency was fully recognized by the New York legislature in the grant of power to the health officer of the port "in the presence of immediate danger to take the responsibility of applying such additional measures as may be deemed indispensable for the protection of the public health."<sup>97</sup> An occasion arose for the exercise of this emergency power in the summer of 1892 when steamers arrived at the port of New York with cases of cholera. The health officer took the "additional measures" and was held by the court<sup>98</sup> to be within his power in landing persons upon Fire Island, within the jurisdiction and contrary to the orders of the local board, on the ground that as a state officer he was empowered to exercise his discretion in an emergency and land passengers temporarily outside the limits prescribed by statute for the quarantine establishment.<sup>99</sup>

Forfeiture of a vessel as a means of enforcement of quarantine was not often included in the law, although it is found in an early

<sup>93</sup> N. Y. St. 1893, ch. 661, sec. 113; 1897, ch. 378, sec. 1210.

<sup>94</sup> N. Y. St. 1866, ch. 74, sec. 16.

<sup>95</sup> *Brown v. Purdy*, 8 N. Y. St. Rep. 143, 1886; cf. *Tormey v. Mayor*, 12 Hun 542, 1878.

<sup>96</sup> *Ex parte Smith*, 146 N. Y. 68, 1895; *Smith v. Emery*, 11 App. Div. 10, 1896.

<sup>97</sup> N. Y. St. 1863, ch. 358, sec. 37; 1892, ch. 486, sec. 13.

<sup>98</sup> *Young v. Flower*, 22 N. Y. Supp. 332, 1893.

<sup>99</sup> In order that there might be no mistake as to the power of the state officials to use force if necessary in the execution of the quarantine we find in old South Carolina laws that incoming vessels were required to anchor "under the guns of the fort" (1759, ch. 881); that small forts should be erected at Beaufort and Georgetown for the better enforcement of quarantine (1783, ch. 1194); that the governor was empowered to employ "boats and able men well armed to enforce quarantine" (1797, ch. 1672); that the quarantine officers were to board vessels for examination "by force of arms;" and that any vessel attempting to evade the quarantine laws was to be fired upon (1809, ch. 1944); *infra*, p. 123.

Virginia law;<sup>100</sup> but forfeiture of goods was common, particularly coffee, cotton, and hides. Such goods brought into the city of New York contrary to the law might be seized and sold by the commissioners of health,<sup>101</sup> and the expenses of removing a vessel from the wharves to quarantine upon order of the mayor or health commissioner formed a lien upon the vessel.<sup>102</sup>

But summary powers, while most essential in regard to quarantine, were also necessary for the abatement of nuisances. "If civil authorities were obliged to wait the slow progress of a public prosecution, the evils arising from nuisances would seldom be avoided."<sup>103</sup> Whether or not a common nuisance could be abated by anyone seems to have been a doubtful point. According to Blackstone<sup>104</sup> a public or common nuisance as "an annoyance to all the King's subjects" must be referred "to the class of public wrongs or crimes and misdemeanors," but while common nuisances "are indictable only, and not actionable"<sup>105</sup> a private person suffering extraordinary damage might abate summarily. Yet in New York in 1832 it was held to "be settled doctrine" that "any person may abate a common nuisance." "A common nuisance is an unlawful act whereby the whole community is injured; all, therefore, are aggrieved, and all have a right to abate it."<sup>106</sup> It was further held that a city as a corporate person had both the right of any person to abate and also the rights given by statute. Such statutory grants appear at an early day.<sup>107</sup> Health authorities were endowed with summary powers over nuisances by statute in Massachusetts and New York at about the same time. In Massachusetts<sup>108</sup> it was made the duty of town boards of health or health officers to remove filth; if on private property, upon failure of the owner to remove the nuisance within twenty-four hours after being so ordered.

<sup>100</sup> Va. St. 1722, ch. 2.

<sup>101</sup> N. Y. St. 1800, ch. 120; 1801, ch. 86; 1811, ch. 175; 1820, ch. 229; 1897, ch. 378, secs. 1207-9.

<sup>102</sup> N. Y. St. 1857, ch. 412; 1893, ch. 661, sec. 121.

<sup>103</sup> Van Wormer v. Mayor, 15 Wend. 263, 1836.

<sup>104</sup> Blackstone's *Commentaries*, 3, p. 216.

<sup>105</sup> *Ibid.*, 4, pp. 161, 167.

<sup>106</sup> Hart v. Mayor Albany, 9 Wend. 571, p. 608; Meeker v. Van Renssalaer, 15 Wend. 397.

<sup>107</sup> The first seems to have been in South Carolina in 1764, to the city of Charleston, by which the authorities were given power to abate nuisances caused by filth in the streets and over drainage and sewage, although in Charleston as early as 1692 nuisances of swine and goats on the streets might be summarily removed by anyone and in 1698 a general power to order the removal of slaughter-houses and other nuisances was given to "the Commissioners and two others" (S. C. St. 1764, ch. 927; 1692, ch. 86; 1698, ch. 162).

<sup>108</sup> Mass. St. 1797, ch. 16.

This is the substance of the present law in Massachusetts; the provisions have remained practically unchanged for more than a hundred years as to the examination and removal of nuisances caused by filth.

In New York City health authorities had power to fill in low lots upon the refusal of the owner,<sup>109</sup> and to abate nuisances caused by stagnant water, lack of drainage, or sewage, and offensive vaults whenever they thought it "necessary for the more speedy execution of the said by-laws," and to destroy putrid substances at any time.<sup>110</sup> In the law of 1820 the power was vague, but in that of 1823<sup>111</sup> the board of health was "authorized to give all directions, and to adopt all such measures" as were necessary to cleanse noisome places, and "to do or cause to be done everything in relation thereto" proper for the preservation of the public health. This continued substantially unchanged until 1850,<sup>112</sup> when the widest powers for summary abatement of nuisances were given to the mayor and council acting as a board of health. Even the act of 1866<sup>113</sup> creating the Metropolitan Board of Health did not give quite such wide summary powers, since by the latter act peremptory abatement without preliminary orders could be used only in time of pestilence. The only additional power conferred on the Metropolitan Board<sup>114</sup> was in extending the power previously granted with regard to nuisances to include the enforcing of repairs on buildings; but this is not included in powers of boards of health in the state at large.<sup>115</sup>

The first permanent statutory grant of powers over nuisances to local authorities generally in New York in 1850 was to make regulations and did not include the power of summary abatement; it was so construed by the courts.<sup>116</sup> The deficiency was remedied in the law of 1867,<sup>117</sup> however, which gave local boards power to issue orders for the suppression of nuisances, and, in case of refusal or neglect to obey the orders, to abate at the expense of the owner. As the law of 1850 did not create boards of health corporate persons they could not take advantage of the decisions in *Hart v. Mayor and*

<sup>109</sup> N. Y. St. 1798, ch. 65.

<sup>110</sup> N. Y. St. 1799, ch. 70.

<sup>111</sup> N. Y. St. 1823, ch. 71.

<sup>112</sup> N. Y. St. 1850, ch. 275, tit. III.

<sup>116</sup> N. Y. St. 1850, ch. 324; *Reed v. P.*, 1 Park Cr. 481, 1854; *Rogers v. Barker*, 31 Barb. 447, 1860.

<sup>117</sup> N. Y. St. 1867, ch. 790.

<sup>113</sup> N. Y. St. 1866, ch. 74.

<sup>114</sup> N. Y. St. 1866, ch. 686, sec. 3.

<sup>115</sup> *Eckhardt v. Buffalo*, 19 App. Div. 1.

*Van Wormer v. Mayor*,<sup>118</sup> in which it has been said that a city council as a corporate person had the right to abate a common nuisance as a party aggrieved. In cases brought against the Metropolitan Board in New York City, however, it was held that the law of 1866 conferred common law rights upon the board of health,<sup>119</sup> i. e., for summary abatement; that the suppression of a nuisance by a board created for that purpose was less objectionable than when done by a private party;<sup>120</sup> and that: "If summary abatement of a public nuisance . . . by a citizen . . . at common law was 'due process of law,' then the execution of an order of the board of health was 'due process,' though its execution might deprive the plaintiff of property without compensation;"<sup>121</sup> and in a more recent case:<sup>122</sup> "The right of summary abatement of nuisances without judicial process or proceeding was an established principle of the common law long before the adoption of our constitution, and it has never been supposed that this common-law principle was abrogated by the provision for the protection of life, liberty, and property in our state constitution although the exercise of the right might result in the destruction of property."<sup>123</sup> Yet in spite of these statements by various courts regarding the right of anyone, including health officials, to abate a public nuisance summarily, and in spite of the legislative grant of "common-law power to abate" to the board of health, there seems to have been a general misunderstanding upon the whole subject, as it seems there never was such a common-law power. In a case<sup>124</sup> involving a bridge declared to be a public nuisance, the court said that "no one has the right to abate it, or sustain an action for damages, unless he has himself sustained some damages not sustained by the rest of the community;" and in *Lawton v. Steele*:<sup>125</sup> "The best considered authorities in this country and England now hold that a public nuisance can only be abated by an individual

<sup>118</sup> *Hart v. Mayor Albany*, 9 Wend. 571, 608, 1832; *Van Wormer v. Mayor Albany*, 15 Wend. 263, 1838.

<sup>119</sup> *Cooper v. Schultz*, 32 How. Pr. 10, 1866; see also *Gardner v. Bd. H.*, 10 N. Y. 409, 1852; *P. v. Supervisors Monroe Co.*, 18 Barb. 567, 1854.

<sup>120</sup> *Weil v. Schultz*, 33 How. Pr. 7, 1866.

<sup>121</sup> *Coe v. Schultz*, 47 Barb. 64, 1866.

<sup>122</sup> *Cartwright v. Cohoes*, 165 N. Y. 631, 1901; affirming 39 App. Div. 69.

<sup>123</sup> See also *License Tax Case*, 5 How. (U. S.) 504.

<sup>124</sup> *Fort Plain Bridge Co. v. Smith*, 30 N. Y. 44, 62, 1864.

<sup>125</sup> *Lawton v. Steele*, 119 N. Y. 226, 1890.



where it obstructs his private right, or interferes at the time with his enjoyment of a right common to many as the right of passage upon the public highway, and he thereby sustains a special injury," although statutory grants of summary powers to boards of health are upheld.

It appears, therefore, that special orders of a board of health for the abatement of special nuisances may be enforced in either one of four ways: (1) by punishment, either as for a misdemeanor or by suit for penalties upon failure to obey the order, but only where the power to attach penalties to special orders has been conferred by statute;<sup>126</sup> (2) by application to the courts to restrain by injunction the continuance of the nuisance; (3) by abatement by the board, after notice either with or without the opportunity for a hearing; and (4) by summary abatement without either notice or hearing.

### 3. *Expenses of Abatement.*

In any abatement by the board expenses are incurred in the process of abatement and the question at once arises, upon whom such expenses should fall. In New York at one time the theory prevailed that the city should pay the expenses of the removal of noxious trades,<sup>127</sup> but within a short time this theory was abandoned in favor of one which compelled the repayment by the owner of expenses for removal of such nuisances as well as those arising from filth, sometimes out of a fine imposed for misdemeanor,<sup>128</sup> or more commonly, recovered by the board of health on suit; if there were in fact no nuisance the expenses cannot be recovered.<sup>129</sup> The latter method has been the one constantly in vogue in Massachusetts for all nuisances except those arising from slaughter-houses.<sup>130</sup> Nuisances occurring in public places, streets, alleys, and wharves have always been removed at the expense of the city,<sup>131</sup> except that in New York City, since the passage of the Metropolitan Health Act,<sup>132</sup> they are to be removed at the expense of the person under contract to keep the streets clean.

<sup>126</sup> *H. Dept. v. Knoll*, 70 N. Y. 530, 1877; *supra*, p. 86.

<sup>127</sup> N. Y. St. 1797, ch. 38; *supra*, p. 8, and n. 41, p. 90.

<sup>128</sup> N. Y. St. 1798, ch. 65.

<sup>129</sup> For New York City, N. Y. St. 1801, ch. 86; 1850, ch. 275; for local boards, 1867, ch. 790; *Haag v. Mt. Vernon*, 41 App. Div. 366, 1899.

<sup>130</sup> For Boston, Mass. St. 1797, ch. 10; for towns, 1797, ch. 16; for cities, 1849, ch. 211.

<sup>131</sup> N. Y. St. 1801, ch. 86; Mass. St. 1797, ch. 16.

<sup>132</sup> N. Y. St. 1866, ch. 74, sec. 14, part 2; 1897, ch. 378, secs. 1176, 1276.



The expenses incurred in the filling of low lots and stagnant pools in New York City have since the first law upon the subject<sup>133</sup> been borne partly by the city and partly by the property benefited; in Massachusetts also expenses for abating nuisances from swamp lands have been assessed, together with the damages and benefits, upon the property affected.<sup>134</sup> These provisions seem to imply economic advantages quite as great as those to health and an exercise of eminent domain along with the police power.

In regard to the efficacy of these various methods of enforcement it is interesting to notice that in the third annual report of the counsel for the Metropolitan Board of Health, 1868, it is stated that while at first the board undertook to abate nuisances summarily, they later found the method of sending notice (orders) to offenders that suit for penalties would be brought in fifteen days unless the nuisance was abated quite as effective and less expensive than the method of summary abatement.<sup>135</sup> The courts, however, have held<sup>136</sup> that where the law gave power to a board of health to make both general and special orders, but provided no penalty for disobedience to the special orders, the board could not impose a penalty but must abate the nuisance and collect the costs.

The experience of the Metropolitan Board coincides with the theory of the courts that for ordinary nuisances summary abatement is not the best method. The reason for caution on the part of health officers is readily found in the fact that anyone abating a nuisance, whether under the disguise of authority conferred by law or not, will be held liable unless the nuisance is one in fact, and the question of the existence of the nuisance is one which must be determined by a court; in other words, any person who abates a nuisance does so at his own risk.<sup>137</sup> The attitude of the courts toward summary abatement is fairly well stated in an opinion<sup>138</sup> in which the court, while allowing the power of the board of health to abate nuisances summarily and sue for expenses, said that the power should be exercised only in extreme cases, and only then when the party charged had had an opportunity to be heard and had failed to show cause why the nuisances should not

<sup>133</sup> N. Y. St. 1799, ch. 70.

<sup>134</sup> Mass. St. 1868, ch. 160.

<sup>135</sup> *Third Annual Report Met. Bd. H.*

<sup>136</sup> *H. Dept. v. Knoll*, 70 N. Y. 530, 1877.

<sup>137</sup> *Infra*, p. 108, "Liability."

<sup>138</sup> *P. v. Wood*, 62 Hun 131, 1891.

be abated, and again:<sup>139</sup> "Whoever abates an alleged nuisance and thus destroys or injures private property, or interferes with private rights, whether he be a public officer or a private person, unless he acts under the judgment or order of a court having jurisdiction, does it at his peril and when his act is challenged in the regular judicial tribunals, it must appear that the thing abated was in fact a nuisance."

### C. REMEDIES (AS AGAINST THE HEALTH AUTHORITIES).

In considering the remedies allowed the individual against hasty or ill-judged action on the part of health officers or boards, it should be noted that they depend less upon definite legislative action than upon general principles deduced and expounded by the courts.

#### 1. *Appeal.*

Since there is no common-law right of appeal from administrative orders and as boards of health are at most only quasi-judicial bodies, the basis of the right of appeal must be found in some statutory grant. In New York appeal from orders of the health officer of the port in regard to quarantine has been allowed by statute ever since 1839<sup>140</sup> in which year the mayor, resident physician, and commissioner of health were constituted a board of appeal with power to grant relief as might appear to the board expedient or proper. This or a similar provision was continued in the quarantine laws of 1850, 1856, 1863, and 1892.<sup>141</sup> No such specific right of appeal is granted by statute in regard to orders for the removal of nuisances; although since the enforcement of orders can be had only through the courts, except in case of summary enforcement by the board itself when the fact of nuisance is open to determination by the court, and since the matter must be brought into court by the board either by indictment or suit, ample opportunity is given for "judicial review" which takes the place of and is equivalent to appeal.

In Massachusetts, while appeal from judgments of a justice of the peace in cases of nuisance was allowed by statute for the city of Boston in 1799,<sup>142</sup> from orders of a jury of inquiry for the removal of noxious

<sup>139</sup> *P. v. Bd. H. Yonkers*, 140 N. Y. 1, 1893; *Haag v. Mt. Vernon*, 41 App. Div. 366, 1899.

<sup>140</sup> N. Y. St. 1839, ch. 359.

<sup>141</sup> N. Y. St. 1850, ch. 275; 1856, ch. 147; 1863, ch. 358; 1892, ch. 486.

<sup>142</sup> Mass. St. 1799, ch. 10, sec. 3.

trades in 1801,<sup>143</sup> and from decisions of justices of the peace in suits to recover penalties for violations of regulations as to both quarantine and nuisances in Boston in 1816,<sup>144</sup> specific right of appeal from orders of boards of health does not appear until 1855<sup>145</sup> in regard to nuisances caused by offensive trades, and then the appeal was from the order of the board to a jury of inquiry, impaneled in the same manner as for highways, which might alter, confirm, or annul the order of the board. Appeal has also been allowed in drainage cases to the county commissioners<sup>146</sup> and to the state board from requirements of local police as to ventilation and plumbing.<sup>147</sup>

In a case<sup>148</sup> arising under the law of 1871<sup>149</sup> for the regulation of slaughter-houses and noxious trades by the state board, in which no right of appeal was expressly given in the statute from an order made after notice and hearing, the court read into the statute the same right of appeal from orders of the state board as was granted by earlier statute<sup>150</sup> from orders of local health authorities. But in a later case<sup>151</sup> the appeal granted by a statute of 1897<sup>152</sup> from orders of the state board of health in regard to water supply was held not to apply to general regulations made by that board under the same law.

## 2. *Injunction against the Health Board or Officer.*

Another remedy used in New York to postpone or prevent the action of boards of health and health officers, especially to prevent the summary abatement of nuisances, is that of injunction. This remedy is not found in any statute but was allowed by the court from early days. Where boards of health or municipal corporations exceed their powers the court will issue an injunction to restrain them.<sup>153</sup> The only known attempt to limit the remedy by injunction is in the

<sup>143</sup> Mass. St. 1801, ch. 16.

<sup>144</sup> Mass. St. 1816, ch. 44.

<sup>145</sup> Mass. St. 1855, ch. 391; R. L., ch. 75, sec. 95; ch. 101, sec. 3.

<sup>146</sup> Mass. St. 1866, ch. 211; 1868, ch. 160, sec. 8; R. L., ch. 75, secs. 80-84.

<sup>147</sup> Mass. St. 1907, ch. 499.

<sup>148</sup> *Sawyer v. Bd. H.*, 125 Mass. 182, 1877; *Driscoll v. Taunton*, 160 Mass. 486, 1894.

<sup>149</sup> Mass. St. 1871, ch. 167.

<sup>151</sup> *Nelson v. State Bd. H.*, 186 Mass. 330, 1904.

<sup>150</sup> Mass. St. 1855, ch. 391.

<sup>152</sup> Mass. St. 1897, ch. 510.

<sup>153</sup> *Clark v. Syracuse*, 13 Barb. 32, 1852, to prevent the destruction of a dam; *Hoffman v. Schultz*, 31 How. Pr. 385, 1866, to prevent the removal of market stalls; *Coe v. Schultz*, 47 Barb. 64, 1866; *Babcock v. Buffalo*, 56 N. Y. 268, 1874, to prevent the filling up of a slip; *Golden v. H. Dept.*, 21 App. Div. 420, 1897.

law of 1867 as to the Metropolitan Board of Health;<sup>154</sup> under the terms of this law no preliminary injunction could be granted against the board except by the Supreme Court, after service of at least eight days' notice. In general "the courts do not interfere to restrain municipal authorities from putting their ordinances in force except where the powers delegated to them have been exceeded or where the proofs make it plain and conclusive that the particular building or business establishment in question is not in reality and in fact, although nominally, within the scope and intent of the ordinance,"<sup>155</sup> and where the board has determined with regard to a nuisance "the court will not assume to reverse its action, unless it appears that such action has been arbitrary, oppressive, or repugnant to justice."<sup>156</sup> Where the fact of the nuisance is established the court will not restrain the board from taking summary action to abate.<sup>157</sup>

But while injunctions against boards of health are granted by the courts in New York, in Massachusetts, on the contrary, in the only case coming to notice in which injunction was applied for against a board of health, it was denied:<sup>158</sup> "The jurisdiction conferred is summary . . . and the objects to be attained would often be defeated . . . if orders of a board of health were subject to judicial examination before being carried out." But at the same time the court held that the decision of the board was not final as to the fact of the nuisance, and that those engaged in abating the nuisance did so at their peril, if later it turned out that there was in fact no nuisance. The matter might be litigated either in a suit to recover expenses brought by the board, or in a suit for damages brought by the owner. This Massachusetts doctrine is unusual and would be most unsatisfactory if it were not for the possibility of appeal. In theory, at least, the New York doctrine offers greater protection to individual rights. In the case of *Stone v. Heath* quoted above, there was no opportunity for appeal, since this case fell under a general regulation; and the appeal allowed by the law of 1897<sup>159</sup> was from quasi-judicial orders, not from quasi-legislative regulations.

<sup>154</sup> N. Y. St. 1867, ch. 956, sec. 9.

<sup>155</sup> *Cushing v. Bd. H. Buffalo*, 13 N. Y. St. Rep. 783, 1887; *Babcock v. Buffalo*, 56 N. Y. 268, 1874.

<sup>156</sup> *Egan v. Bd. H.*, 9 N. Y. App. Div. 431, 1897.

<sup>157</sup> *Cartwright v. Cohoes*, 39 App. Div. 69, 1899; affirmed, 165 N. Y. 631, 1901.

<sup>158</sup> *Stone v. Heath*, 179 Mass. 385, 1901.

<sup>159</sup> Mass. St. 1897, ch. 510.



The present tendency, therefore, in New York to belittle the quasi-judicial aspects of boards of health and to insist upon the administrative character of their actions is clearly shown by the lack of provisions for appeal from their orders and by the frequent use of injunctions to postpone or prevent action under their orders, particularly to prevent the summary abatement of nuisances; the tendency in Massachusetts, on the contrary, to emphasize the quasi-judicial aspect of boards of health is shown by the constant use of appeal, and by the denial of injunctions against them: the dignity of the board is sustained. In *Peebles v. Boston*<sup>160</sup> the court said: "The action of the board of health . . . is judicial in its character, and an order made by the board and not appealed from, has the binding force and effect of a judgment by a tribunal of competent jurisdiction," and notice of an appeal from its orders must be given to the board. In *Nelson v. State Board of Health*<sup>161</sup> the court drew a sharp distinction between the quasi-legislative and quasi-judicial acts of boards of health, holding that appeal might be had from the latter only.

### 3. *Liability.*

Summary abatement of nuisances, whether by individuals, boards of health, or health officers, would, of course, be open to grave abuses unless there were some means of holding in check a too zealous officer, some drawback to a free use of such power by boards. Such drawback or limitation is found in the liability of the officer or board in case of abuse of the power granted. The statutes have been generally silent in regard to liability for damage or destruction to property under the guise of benefit to the public health; the protection of individual rights, therefore, has depended upon court decisions. Any person abating a private nuisance was liable at common law in an action for trespass or damages unless the nuisance were one in fact; so, too, the New York courts have held that although officers or boards of health were given authority by statute to abate nuisances summarily, they acted at their own risk unless the nuisance were one in fact, and the owner had always a right to bring the question of fact in some way before the courts. An instance of this occurred in the case of Under-

<sup>160</sup> *Peebles v. Boston*, 131 Mass. 197, 1881.

<sup>161</sup> *Nelson v. State Bd. H.*, 186 Mass. 330, 1904.



wood *v.* Green<sup>162</sup> in which it was held that a city inspector, as agent of the board, could not justify the removal of dead hogs from a train under authority of a city ordinance for the removal and destruction of putrid and unsound beef and pork unless they were shown to be a menace to the public health, that he must prove the facts tending to invoke the exercise of his discretion. In *Smith v. Emery and ex parte Smith*<sup>163</sup> an action for false imprisonment was maintained against a health commissioner who had quarantined an expressman upon his refusal to be vaccinated; the court held that the statutory power to isolate was dependent upon actual infection or exposure to contagious disease. But where the infection was a fact, although the decision as to the existence of that fact was made by the officer, summary removal to a pest-house was upheld.<sup>164</sup>

Holding an officer personally liable for damages incurred in abating nuisances is not conducive to a rigid enforcement of the law, hence a few attempts have been made in New York to throw the onus of liability upon the board of health officially or upon the municipality which they represent, rather than upon the individuals composing the board. The amendment of 1867 to the Metropolitan Health Act<sup>165</sup> made such an attempt, the same provisions being continued in the New York City charter of 1882 and in the charter of Greater New York in 1897.<sup>166</sup> This provision applies only to boards in New York City and is "plainly limited to immunity for unjust or illegal acts done in good faith or with ordinary discretion;"<sup>167</sup> there is no provision freeing the members of boards of health in the state at large from personal liability.<sup>168</sup>

In Massachusetts the courts have uniformly held that the members of a board of health are liable in damages for actions which were *ultra vires*, or where the facts as to the nuisance upon which the action was based are successfully questioned. The cases which have arisen have been chiefly in connection with the seizure and use of houses for smallpox patients without a warrant from a justice of the peace.<sup>169</sup>

<sup>162</sup> *Underwood v. Green*, 42 N. Y. 140, 1870.

<sup>163</sup> *Smith v. Emery*, 11 App. Div. 10, 1896; *ex parte Smith*, 146 N. Y. 68, 1895.

<sup>164</sup> *Brown v. Purdy*, 8 N. Y. St. Rep. 146, 1886.

<sup>165</sup> N. Y. St. 1867, ch. 956, secs. 6-13.

<sup>166</sup> N. Y. St. 1882, ch. 410, secs. 595, 599; 1897, ch. 378, sec. 1196.

<sup>167</sup> *Sbarboro v. H. Dept.*, 26 App. Div. 226, 1898.

<sup>168</sup> *Bamber v. Rochester*, 26 Hun 587, 1882.

<sup>169</sup> *Spring v. Hdy Park*, 137 Mass. 554, 1884; *Brown v. Murdock*, 140 Mass. 314, 1885; *Hersey v. Chapin*, 162 Mass. 176, 1894; *Barry v. Smith*, 191 Mass. 78, 1906.

Parallel to these cases are those of domestic animals killed on account of contagious diseases. In a case<sup>170</sup> of the latter kind a local board of health was held liable for killing, at the order of the commissioner of contagious diseases of animals, a horse judged to have glanders, when it was held by the court to be proved that the horse did not have glanders. Later the law upon the subject was amended and compensation was allowed for animals so killed.<sup>171</sup> This latter would seem to be the correct theory for the destruction of property as a nuisance.<sup>172</sup>

#### 4. *Compensation.*

Provision for compensation to the owners for damages or expenses incurred in the removal of nuisances is found in a few laws. In New York in 1796<sup>173</sup> the law provided for reasonable compensation to be paid owners of noxious trades for the expenses incurred for removal upon orders of the health authorities, the amount of the compensation to be agreed upon between the owner and mayor, or, in case they failed to reach an agreement as to the amount, by a jury appointed for that purpose. A similar provision is found in the law of 1797<sup>174</sup> but not after 1798.<sup>175</sup> Compensation was also provided for private buildings used as temporary hospitals during an epidemic,<sup>176</sup> and in 1895 for buildings ordered destroyed by the board of health.<sup>177</sup> In Massachusetts compensation was provided in the Revised Statutes of 1836<sup>178</sup> to be paid by towns to the owners of houses impressed as hospitals for contagious disease, although the courts have held the towns not liable unless a warrant had been obtained according to the terms of the law.<sup>179</sup> By the law of 1855<sup>180</sup> when noxious trades were forbidden in a given locality by the local board of health an appeal to a jury was allowed; pending the decision of the jury the trade must be suspended, and in case the decision of the jury annulled the order of the board of health damages for the loss occasioned by the suspension

<sup>170</sup> *Miller v. Horton*, 152 Mass. 540, 1801.

<sup>171</sup> Mass. St. 1802, ch. 105, sec. 3.

<sup>172</sup> Freund, *Police Power*, secs. 521, 524, 602.

<sup>176</sup> N. Y. St. 1850, ch. 275.

<sup>177</sup> N. Y. St. 1805, ch. 567; H. Dept. v. Dassori, 159 N. Y. 245, 1809.

<sup>178</sup> Mass. R. S., ch. 21, sec. 24.

<sup>179</sup> *Spring v. Hyde Park*, 137 Mass. 554, 1884.

<sup>180</sup> Mass. St. 1855, ch. 391; Mass. R. L., ch. 75, secs. 96-98.

<sup>173</sup> N. Y. St. 1796, ch. 38, secs. 12, 13.

<sup>174</sup> N. Y. St. 1797, ch. 16.

<sup>175</sup> N. Y. St. 1798, ch. 65.

of his business might be recovered by the owner from the city or town. This is continued in the present laws. Compensation has been provided for clothing or furniture destroyed at the order of the board of health;<sup>181</sup> also when wet lands are deemed a nuisance and ordered filled or drained,<sup>182</sup> although in this instance a hearing must be given by the board of health for the apportionment of damages and assessments for expenses, and the whole process is more like an exercise of the power of eminent domain than that of the police power.<sup>183</sup> Within the past two years the legislature of Massachusetts has taken very advanced ground by providing for compensation to wage-earners placed under local quarantine by the city or town board of health; such compensation to reach the extent of three-fourths of the regular wages, provided the amount did not exceed two dollars a day.<sup>184</sup>

The object of the various remedies against the health authorities is, of course, the preservation of the rights of the individual both in his property and in his person. So long as there is no great stress of immediate danger the liberty of the individual is fairly well guarded by the remedies provided in the laws, but immediately upon any occasion of danger or panic the executive officers of the law are likely to carry matters with a high hand, as witness the forcible ejectment of people from their houses in New York City during the yellow fever epidemic in 1819;<sup>185</sup> the acts of the health committee during the epidemic of cholera in New York City in 1849;<sup>186</sup> and the arbitrary dealings of the health officer of the port of New York with passengers from Europe during the cholera scare in 1892.<sup>187</sup> The American people are long suffering. In connection with the last-named cholera scare a prominent member of the New York health department was asked what had become of personal liberty; his answer showed the regard with which all the officials viewed the subject: "Personal liberty, your grandmother!"

<sup>181</sup> Mass. St. 1903, ch. 306.

<sup>182</sup> Mass. St. 1868, ch. 160; P. L., ch. 75, secs. 75-85.

<sup>183</sup> Compensation is also provided in both New York and Massachusetts for the slaughter of cattle supposed to be tuberculous, Mass. St. 1892, ch. 195; N. Y. St. 1892, ch. 487; Freund, *Police Power*, sec. 524.

<sup>184</sup> Mass. St. 1906, ch. 225.

<sup>186</sup> *Supra*, p. 15.

<sup>185</sup> *Supra*, p. 19.

<sup>187</sup> *The Review of Reviews*, 6, pp. 262, 343, 393, 654, 729.

Throughout the two hundred years of health legislation the pendulum has swung backward and forward; no health laws were passed during the first three-quarters of that time without the impetus of epidemic contagious disease; after a visitation of such disease the powers of the officers were increased, the sanctions made more severe; with the passing of the epidemic, and the enjoyment of a period of immunity the unnecessary harshness of the measures became apparent and amelioration of conditions came about either by less strict administration of the laws or through modifying legislation; such modifications, however, cut off only the unnecessary provisions which had been the result of panic, a substantial residuum of reasonable power was always left as a foundation for future laws. Then another epidemic, the need of more power, a new law.

It has been only within the past thirty years that a forehanded attitude of scientific preparedness has been observed, that officials and legislators alike have [realized that constant watchfulness is the price of immunity from disease, as for individuals, so for the state.

The same swinging of the pendulum although in a less marked degree is to be noticed in the decisions of the courts; in time of pestilence or threatened danger laws conferring powers on health officials have not received a strict construction; witness the decisions in *Hart v. Albany* and *Van Wormer v. Albany* in 1832 and 1836.<sup>188</sup> Indeed, it is doubtful whether the laws establishing the Metropolitan Board in 1866 would have been so well sustained had it not been a time of great danger from cholera; and the latest opinion in regard to the summary powers of a board of health was certainly influenced by the fact that cholera was again knocking at the quarantine door of the port of New York.<sup>189</sup> Judges are but men; they must take cognizance of the existing conditions, the prevailing sentiments, and they interpret the laws accordingly.

The main object of the organization of government under our democratic theory is the protection and welfare of the people; not the least item in the sum of that welfare is public health. In the city of Chicago the first incorporation of the municipality (August 5, 1833, under the

<sup>188</sup> *Hart v. Albany*, 9 Wend. 571, 1832; *Van Wormer v. Albany*, 15 Wend. 263, 1836.

<sup>189</sup> *Supra*, p. 96, n. 75.

general law) came about through the desire of the inhabitants to protect themselves from a recurrence of the invasion of cholera in 1832,<sup>190</sup> and among the earliest ordinances adopted by the new board of trustees were some for the protection of health. Without private health the individual is useless, without protection of the public health government is a failure. *Salus populi suprema lex.*

<sup>190</sup> Ill. St. 1 Mar. 1831; Kirkland, *History of Chicago*, 2, p. 282; *Reports of the Chicago Board of Health*, 1.



## APPENDIX A.

It has been quite impossible to give a detailed history of the legislation with regard to health in all the states of the Union. The history of health legislation in New York and Massachusetts has been considered very fully because they were the two maritime states containing the most important harbors, because they have had many and complete laws along sharply contrasting lines, and because the legislation of one or the other has been frequently adopted by other states; both South Carolina and Louisiana have copied New York health laws verbatim, and other New England states have enacted laws very similar to those of Massachusetts. It is not therefore essential to consider in detail the history of legislation in any other state. It has seemed well, however, to add a summary of federal legislation and of that of the two important maritime states of Pennsylvania and South Carolina, both of which enacted laws in regard to health at a very early day.

### LEGISLATION IN PENNSYLVANIA.

Philadelphia, like New York, suffered in early days from visitations of yellow fever. The first severe epidemic known to be yellow fever, but called "Barbadoes distemper," occurred in 1699 and carried off 220 from a population of 3,800.<sup>1</sup> This epidemic was followed by the first quarantine law to be found on the statute books of North America, that of 1700.<sup>2</sup> It was entitled "An Act to Prevent Sickly Vessels Coming into this Government." "Whereas it hath been found by sad experience that the coming and arriving of unhealthy vessels at the ports and towns of this province and territories, and the landing of their passengers and goods before they have lain some time to be purified, hath proven very detrimental to the health of the inhabitants of this province:" it was enacted that "No unhealthy or sickly vessels coming from any unhealthy or sickly place whatsoever, shall come nearer than one mile to any of the towns or ports of this province or territory, without bills of health," with a heavy penalty imposed upon any one landing goods or passengers from such vessels until a license

<sup>1</sup> Gamgee, *Yellow Fever, A Nautical Disease*.

<sup>2</sup> Pa. St. 1700, ch. 62.

had been obtained from the governor and council for those landing at Philadelphia, or from two justices of the peace for other ports. Another epidemic of yellow fever in 1741 was probably the occasion of the two laws of the following year:<sup>3</sup> providing a hospital for infected passengers, and making it the duty of the collector of a tax imposed on imported convicts to report cases of disease to the governor who might send them to the hospital. An act was passed in 1774<sup>4</sup> "to prevent infectious diseases being brought into this province," but it has not been possible to obtain a copy of this law, nor of the supplements which followed it in 1783, 1793, and 1794. The last two were temporary and were directly caused by the frightful epidemic of yellow fever<sup>5</sup> which devastated Philadelphia in the summer and early fall of 1793 in which there were over 4,000 deaths. In April, 1794,<sup>6</sup> there was, however, a permanent and elaborate act for the establishment of a health office and for otherwise securing the city and port of Philadelphia from the introduction of pestilential diseases. This law provided for a health office to consist of suitable buildings for a hospital, dwellings, offices, and warehouses upon State Island; for the appointment by the governor of a resident physician to reside upon the island and to visit and examine all incoming vessels, a consulting physician, and a health officer to keep an office in the city at which the bills of health given by the resident physician were to be presented. The law also provided for the selection by the mayor and aldermen of Philadelphia and justices of the peace of the Northern Liberties and Southwark of a board of 24 inspectors, 14 from the inhabitants of Philadelphia, and five each from the Northern Liberties and Southwark. The board of inspectors was to make such regulations as were necessary for carrying the act into effect, to appoint a steward, matron, and nurses and attendants as needed. The law also prescribed in detail the duties of these various officers, the measures to be taken in case of disease, and the amount of space and cleansing required of all ships bringing immigrants. The governor was empowered to proclaim quarantine against any port whether of the United States or a foreign

<sup>3</sup> Pa. St. 1742, chs. 354, 357.

<sup>4</sup> Pa. St. 1774, ch. 689; supps. 1783, ch. 1023; 1793, ch. 1603; 1794, ch. 1708.

<sup>5</sup> Carey, M., *Short Account of Malignant Fever Lately Prevalent in Phila.*; Webster, *History of Epidemic Diseases*.

<sup>6</sup> Pa. St. 1794, ch. 1747.

country in which there was pestilential or contagious disease, small-pox and measles excepted. The expenses of quarantine were to be paid by the persons affected or by the masters of the vessels in which they had come; further expenses of the health office were to be met by a tax raised with the other taxes of the city and county. Amendments,<sup>7</sup> shown to be necessary by the return of yellow fever in 1794 and 1797, changed the method by which the board of inspectors performed their duties, enabled them to lay a tax for the support of the health office, and otherwise increased their power. As so amended the law of 1794 is at the foundation of the quarantine laws of Pennsylvania as they exist on her statute books to the present time.

A new quarantine act in 1799,<sup>8</sup> following an epidemic of yellow fever in 1798 somewhat worse than in the preceding year, repealed the inconsistent parts of the earlier acts, provided a new method of nominating the board, now incorporated and called the board of health, and extended the boundaries of the district over which the law was operative. Quarantine provisions were even more detailed and the board was given power to prevent all communication by land or by sea with places in the United States at which contagious disease existed, as also to quarantine any part of the city in which such disease was discovered or to remove and isolate sporadic cases. This act was renewed with minor amendments in 1803 and 1806, with supplements in 1812, 1813, and 1817,<sup>9</sup> and by a new law repealing the previous laws but embodying their substance in 1818;<sup>10</sup> the law of 1818 still stands upon the statute books. There have been a number of supplementary acts covering minor points;<sup>11</sup> the territory over which the board exercised jurisdiction was extended from time to time;<sup>12</sup> and the administration of the law, after the incorporation of the county with the city of Philadelphia in 1854,<sup>13</sup> was in the hands of the city board of health until 1893; but no substantial changes in power have been made, and even in 1893, upon the creation of a state board of quarantine, the law was entitled a supplement to the act of 1818. The law of 1893<sup>14</sup> authorized the governor to suspend the state

<sup>7</sup> Pa. St. 1794, ch. 1778; 1795, ch. 1833; 1795, ch. 1836.

<sup>8</sup> Pa. St. 1799, ch. 2083.

<sup>9</sup> Pa. St. 1803, ch. 2375; 1806, ch. 2670; 1812, ch. 3555; 1813, ch. 3725; 1817, ch. 4368.

<sup>10</sup> Pa. St. 1818, ch. 4483.

<sup>11</sup> Pa. St. 1821, ch. 5042; 1824, ch. 5520; 1827, ch. 5880; 1832, ch. 250; 1850, ch. 220; 1852, ch. 134.

<sup>12</sup> Pa. St. 1848, ch. 21.

<sup>14</sup> Pa. St. 1893, ch. 257.

<sup>13</sup> Pa. St. 1854, ch. 16.

quarantine whenever it was shown to his satisfaction that the federal government was maintaining a sufficient quarantine, but until such suspension the state quarantine was to be administered by a board consisting of the president of the College of Physicians of Philadelphia, the secretary of the state board of health, the president of the Philadelphia Maritime Exchange, the health officer, the quarantine physician, who was to be the executive officer of the board, and two other members, one appointed by the mayor of Philadelphia, and one by the governor. The quarantine legislation of Pennsylvania has been unusual in that it was administered almost from the beginning by an incorporated board with jurisdiction until 1854 over a district consisting of the city of Philadelphia and surrounding villages, and in that the substance of the law of 1794, as continued in that of 1818, is the foundation of the law of the present time; there has been no series of laws, each repealing its predecessor. Local quarantine within five miles of any city has been under the control of city councils since 1874.<sup>15</sup>

There has been very little special legislation in Pennsylvania in regard to smallpox. The fact has been mentioned<sup>16</sup> that in the early laws smallpox and measles were made exceptions to the contagious diseases for which a quarantine might be established; this exception was removed so far as smallpox was concerned in 1824<sup>17</sup> by a law which also forbade inoculation without a special permit from the board of health. A further law in 1849<sup>18</sup> emphasized the law of 1824. There was no legislation with regard to vaccination until 1889<sup>19</sup> when local boards in cities of the third class were empowered to enforce vaccination. In 1895<sup>20</sup> power was given to boards in cities of the second class to require the vaccination of all persons in the city if necessary to prevent the spread of smallpox. In the same year<sup>21</sup> principals of schools in all municipalities were required to exclude unvaccinated children from the schools. Not until 1905<sup>22</sup> was the department of health in "cities of the first class" given power to enforce vaccination and then only for persons connected with the schools or public institutions. Finally in 1907<sup>23</sup> an act creating boards of health in townships gave

<sup>15</sup> Pa. St. 1874, ch. 152.

<sup>16</sup> Pa. St. 1794, ch. 1747; 1799, ch. 2083.

<sup>17</sup> Pa. St. 1824, ch. 5520.

<sup>18</sup> Pa. St. 1849, ch. 271.

<sup>19</sup> Pa. St. 1889, ch. 247.

<sup>20</sup> Pa. St. 1895, ch. 124.

<sup>21</sup> Pa. St. 1895, ch. 258.

<sup>22</sup> Pa. St. 1905, ch. 165.

<sup>23</sup> Pa. St. 1907, ch. 228.



such boards of health power to enforce vaccination of all persons in their townships whenever it was necessary to prevent the spread of smallpox. There was one other law aimed especially against smallpox, that of 1903,<sup>24</sup> occasioned by the prevalence of smallpox and limited to two years; but the provisions of this law were intended rather to enable the state board to enforce local quarantine against smallpox in cases where a local board did not exist or was inefficient, than to confer general or permanent power.

The first legislation in regard to nuisances is a law of 1712<sup>25</sup> "for the better government of Philadelphia," which forbade any person to obstruct the streets with rubbish, filth, or otherwise, with a penalty for failure to remove such nuisances upon notice; the law laid upon constables the duty of reporting such nuisances to the magistrates, with a penalty for neglect, and also gave to the mayor and council power to regulate the shambles. This law, however, did not long survive, as we find that it was "repealed by the Queen in council in 1714." No further legislative action regarding nuisances was taken until 1762,<sup>26</sup> when an act for paving and cleansing the streets and providing for sewers was passed. It was not until after the frightful epidemic of yellow fever in 1793 and the quarrels among the physicians as to its origin<sup>27</sup> that we find any lasting power over nuisances. The law of 1799, enacted primarily for purposes of quarantine, contained a section<sup>28</sup> giving power to the board of health to cause the removal of all offensive and putrid substances and nuisances which might have a tendency to endanger the public health. This section was continued in the law of 1806.<sup>29</sup> In 1812<sup>30</sup> the authority of the board was extended to include the power to enter and search houses and other places suspected of harboring a nuisance upon warrant from a justice after complaint by two householders; to order abatement, and to abate summarily in case of the refusal or neglect of the owner to obey the order of the board. These provisions were included in the law of 1818,<sup>31</sup> the basis of the present law.

<sup>24</sup> Pa. St. 1903, ch. 58.

<sup>25</sup> Pa. St. 1712, ch. 186.

<sup>26</sup> Pa. St. 1762, ch. 480.

<sup>27</sup> Pamphlets published by the Academy of Medicine and College of Physicians of Philadelphia in 1798; also: Caldwell, Rush, Currie and Cathrall, etc.

<sup>28</sup> Pa. St. 1799, ch. 2083, sec. 23.

<sup>30</sup> Pa. St. 1812, ch. 3555, sec. 8.

<sup>29</sup> Pa. St. 1806, ch. 2670, sec. 24.

<sup>31</sup> Pa. St. 1818, ch. 4483.



The powers of the board of health over nuisances have been increased from time to time by supplemental laws; several, noticeably those passed in the years of actual or threatened invasions of cholera, were concerned with the removal and disposition of the contents of offensive vaults;<sup>32</sup> one forbade the storage of large quantities of perishable vegetables;<sup>33</sup> and two others regulated the keeping of hog-pens.<sup>34</sup> Offensive trades were not subjects of legislation until 1855,<sup>35</sup> when bone boiling and other noxious trades were forbidden within the city without a permit from the board of health. Since 1830<sup>36</sup> the decision of the board of health as to the existence of a nuisance has been final, the fact of the nuisance may not be questioned. This unusual doctrine is not only embodied in law in Pennsylvania but the law has been upheld by the Supreme Court of the state.<sup>37</sup> Aside from these supplementary acts the powers over nuisances granted to the board of health by the law of 1818 have not been changed, although administered successively by four differently chosen boards, a bureau, and a department.

Outside of the city of Philadelphia there seems to have been no general power over nuisances given to municipalities, except in the charters of some of the cities, until after the establishment of the state board of health; by the law creating that body<sup>38</sup> it was empowered to order nuisances or causes of disease abated or removed where there was no local board or where the local board was inefficient. The general city law of 1874<sup>39</sup> gave councils in cities of the third class wide powers over nuisances with authority to create local boards by ordinance and endow them with the same powers; the same powers over all health matters were granted to boards of health of boroughs in 1893;<sup>40</sup> and similar powers to bureaus of health in cities of the second class in 1895.<sup>41</sup> In the same year all boards of health were empowered to make regulations concerning house drainage; and in 1897<sup>42</sup> bone boiling was forbidden in any city without a permit from the board of health.

<sup>32</sup> Pa. St. 1826, ch. 5787; 1830, ch. 181; 1848, ch. 21; 1849, ch. 271; 1852, ch. 131; 1855, ch. 98 1860, ch. 374; 1879, ch. 40.

<sup>33</sup> Pa. St. 1821, ch. 5042.

<sup>34</sup> Pa. St. 1849, ch. 271; 1850, ch. 220.

<sup>35</sup> Pa. St. 1855, ch. 415.

<sup>36</sup> Pa. St. 1830, ch. 181.

<sup>37</sup> *Kennedy v. Bd. Health*, 2 Pa. St. 366, 1845; *supra*, pp. 98, 107-8.

<sup>38</sup> Pa. St. 1885, ch. 37.

<sup>39</sup> Pa. St. 1874, ch. 152.

<sup>40</sup> Pa. St. 1893, ch. 42.

<sup>41</sup> Pa. St. 1895, ch. 258.

<sup>42</sup> Pa. St. 1897, ch. 56.

The administration of all laws relating to health in Pennsylvania was for nearly 100 years in the hands of a unique board of health, unique in that it was a body corporate with jurisdiction over a district including the city of Philadelphia and the surrounding villages, in that it had power to lay a tax to meet expenses, and in that it had power over both quarantine and nuisances; the members of the board were appointed by the mayor and aldermen of Philadelphia and the justices of the peace of the villages included from 1799<sup>43</sup> to 1806; in 1806<sup>44</sup> the number was reduced from twelve to five and they were appointed by the governor; in 1818<sup>45</sup> the number was increased to 11 and the members were elected from the various municipalities in the district. This method continued until 1854<sup>46</sup> when, by the enlargement of the city, the whole of the territory over which the board had jurisdiction was included in the city and the members of the board were elected, one from each ward; four years later<sup>47</sup> a new method of selection was tried, the board to consist of 12 reputable citizens, of whom three were to be appointed, one each year, by the judges of the district court, three by the judges of the court of common pleas, three by the judges of the Supreme Court of the state, and three elected by a joint convention of the select and common councils of the city; there was no further change in the method of selection until 1885<sup>48</sup> when the number was again reduced to five to be appointed by the mayor and council, and the board attached to the department of public safety, the director of which was to be the president and chief executive officer with the appointment and control of subordinates; finally in 1899<sup>49</sup> the board of health was abolished and a bureau of health created in the department of public safety. There has been one more change since that time;<sup>50</sup> the creation of a "Department of Public Health and Charities" in charge of a director appointed by the mayor; the director, in turn, has the appointment and entire control of all subordinate officers and employees in the department.

Outside of the city and port of Philadelphia there were no boards of health except as provided in the charters of individual cities<sup>51</sup>

<sup>43</sup> Pa. St. 1799, ch. 2083.

<sup>44</sup> Pa. St. 1806, ch. 2670.

<sup>45</sup> Pa. St. 1818, ch. 4483.

<sup>46</sup> Pa. St. 1854, ch. 16.

<sup>51</sup> Pittsburgh, 1851-2; Harrisburg, 1872; Williamsport, 1872, etc.

<sup>47</sup> Pa. St. 1850, ch. 395.

<sup>48</sup> Pa. St. 1885, ch. 33.

<sup>49</sup> Pa. St. 1899, ch. 11.

<sup>50</sup> Pa. St. 1903, ch. 112; 1905, ch. 165.

until the passage of the general law for the classification of cities in 1874. The care, management, and administration of all matters relating to the public health in cities of the second class were confided to a department of public safety in such cities in 1887;<sup>52</sup> in 1895 a bureau of health was provided by law for all cities of this class.<sup>53</sup> In cities of the third class (less than 100,000 population) the city councils were empowered by the general law of 1874<sup>54</sup> to make regulations to prevent the introduction of contagious diseases and enforce them within five miles of the city, and also for the abatement of nuisances; they were also authorized to appoint boards of health and endow them with suitable powers. A law in 1889<sup>55</sup> further enumerated the powers and duties of such boards of health. Town councils and burgesses of all boroughs were authorized to create boards of health in 1893,<sup>56</sup> and commissioners of townships in 1907.<sup>57</sup>

A state board of health was not established in Pennsylvania until 1885.<sup>58</sup> The board created at that time was given, besides the usual investigative functions, the power to order the abatement and removal of all nuisances and causes of disease and to enforce quarantine in places where there was no local board, or in places where there was a board but where the sanitary laws and regulations were inoperative. Since its creation the state board has also been given power to examine the water supply of Philadelphia.<sup>59</sup>

In 1905 a state department of health was created<sup>60</sup> to consist of a commissioner of health and an advisory board. The quasi-legislative powers of the former board, the making of rules and regulations, were intrusted to the advisory board; all other functions were transferred to the commissioner together with new powers of a quasi-judicial nature—to issue subpoenas to compel witnesses, and issue warrants—and powers for the summary abatement of nuisances. The state was to be divided into 10 districts in each of which there was to be a health officer with supervision and control of the sanitary affairs of the district, and assistants as needed, all appointed by the commissioner. And, most important, “The commissioner of health may

<sup>52</sup> Pa. St. 1887, ch. 263.

<sup>53</sup> Pa. St. 1895, ch. 258.

<sup>54</sup> Pa. St. 1874, ch. 152.

<sup>55</sup> Pa. St. 1889, ch. 247.

<sup>60</sup> Pa. St. 1905, ch. 218.

<sup>56</sup> Pa. St. 1893, ch. 42.

<sup>57</sup> Pa. St. 1907, ch. 228.

<sup>58</sup> Pa. St. 1885, ch. 37.

<sup>59</sup> Pa. St. 1899, ch. 116.

revoke or modify any order, regulation, by-law, or ordinance of a local board of health, concerning a matter, which, in his judgment, affects the public health beyond the territory over which such local board has jurisdiction."

Pennsylvania has therefore gone one step beyond even Massachusetts in centralization of authority over matters pertaining to the public health.

#### LEGISLATION IN SOUTH CAROLINA.

In South Carolina, as in more northern states, legislation with regard to health has followed epidemics of contagious disease and has attempted to prevent the spread of both yellow fever and smallpox. Indeed legislation in behalf of health in South Carolina had reached an advanced stage before the first law upon the subject had been passed in New York. As in Massachusetts and New York, epidemics of smallpox occurred every few years.<sup>61</sup> The first epidemics known to be yellow fever occurred in Charleston in 1693 and 1699, but were called the plague. The fever raged again in 1703, in 1728, when the name of yellow fever was first applied to it, in 1732, 1739, 1745, and in 1748. From 1750 to 1792 the city was almost exempt, but in the 47 years between 1792 and 1839 it reached epidemic proportions no less than 16 times.<sup>62</sup>

The earliest health legislation in South Carolina was for the purpose of preventing nuisances in Charleston. The very first law was one forbidding the nuisance of swine at large in the streets.<sup>63</sup> As early as 1698<sup>64</sup> a law was passed by which power was given to any two of the commissioners to order the filling-up of offensive privies; in another section slaughter-houses, cattle and hog pens, or "any other thing whatsoever" judged a nuisance by the commissioners was to be removed at their order under penalty. There were various renewals and additions to these early acts,<sup>65</sup> and that they were intended not merely for comfort, but to prevent disease is clearly shown in some of the preambles, as in that of the law of 1704 which stated that because of the nuisances arising from slaughter-houses "the air is corrupted and infected, many maladies do happen and

<sup>61</sup> Drayton, Gov. John, *A View of South Carolina in 1802*.

<sup>62</sup> Census of Charleston, 1848, article on public health; Carroll's *Historical Collections*, 2, p. 492.

<sup>63</sup> S. C. St. 1692, ch. 86.

<sup>64</sup> S. C. St. 1698, ch. 162.

<sup>65</sup> S. C. St. 1700, ch. 190; 1704, ch. 231; 1746, ch. 740.



intolerable diseases." By 1764<sup>66</sup> special commissioners for putting it into force were named in a law against nuisances.

A law with regard to quarantine was enacted as early as 1712.<sup>67</sup> This law named a commissioner, gave him power to board incoming vessels, to examine under oath those on board, to order suspected persons sent to a pest-house, and to order to quarantine for 20 days any vessel on which there had been a case or "death from small-pox, Siam distemper, Guinea fever, or other malignant contagious disorder." Heavy penalties were imposed for resisting or hindering the commissioner; negroes or goods landed contrary to his orders were declared forfeited. The activities of the commissioner were confined to the harbor of Charleston; quarantine for other ports was to be enforced at the discretion of the governor. This law of 1712 was limited to two years but was revived in 1714 and 1719.<sup>68</sup> A new law in 1721<sup>69</sup> placed the enforcement of the quarantine provisions in the hands of the commander of the fort under the direction of the governor. This law, with additions in 1744, 1747, and 1752,<sup>70</sup> continued in force until 1759<sup>71</sup> when it was replaced by a new and stringent law which required all incoming vessels to "anchor under the guns of the fort" where an examination under oath was to be conducted by the commander and his permit granted before the vessel could proceed to the city; there a further permit was required from the governor before the master of the vessel could "enter and trade." This was about the time of the first New York legislation. The quarantine law of 1759 expired by its own limitation at the end of five years but was revived in 1783 for a few months to be shortly superseded by a new and similar act in 1784;<sup>72</sup> the last act extended quarantine to all the ports of the state as directed by the governor,

<sup>66</sup> S. C. St. 1764, ch. 927.

<sup>67</sup> S. C. St. 1712, ch. 317. In the Appendix of the *Year Book of Charleston* for 1890 a quotation is given from a law of 1698 for the regulation of pilots and for quarantine, which antedates the Massachusetts and Pennsylvania laws by two years. This law does not appear in the published statutes of South Carolina. The quotation is as follows: "And be it further enacted, that the pylott aforesaid, shall enquire of every Master or Commander of every vessel whether any contagious disease be on his vessel, and the Master or Commander of every vessel shall give a true account thereof; and if there be any contagious sickness on board, the pylott shall acquaint the Master not to come above one mile to the Westward of Sullivan's Island on penalty of Tenn Pounds on the pylott so neglecting; and the penalty of Fifty Pounds on every Master or Commander of any vessel, and not coming to an anchor as aforesaid, after notice given him."

<sup>68</sup> S. C. St. 1714, ch. 342; 1719, ch. 413.

<sup>70</sup> S. C. St. 1744, ch. 720; 1747, ch. 752; 1752, ch. 803.

<sup>69</sup> S. C. St. 1721, ch. 438.

<sup>71</sup> S. C. St. 1759, ch. 881.

<sup>72</sup> S. C. St. 1784, ch. 1219.



and forbade goods or persons to be landed without a bill of health. This law with various amendments,<sup>73</sup> the later ones brought about by fear of Asiatic cholera, continued until 1868<sup>74</sup> when it was largely superseded by a law copying the New York law of 1856. Up to this time quarantine had been performed upon the order of the governor; from the passage of this act vessels from certain places and those in an unsanitary condition were to perform quarantine as a matter of course. Later amendments gave the health officer power to vaccinate all on board when he thought necessary;<sup>75</sup> and extended the penalties of the law to employees of railroads, express companies, and other conveyances<sup>76</sup> for receiving or conveying any person infected with contagious disease.

Meanwhile as early as 1738<sup>77</sup> a special law was passed to prevent the spread of smallpox in Charleston, forbidding inoculation within two miles of the city, and empowering two justices of the peace, the church wardens, and constables (a local board of health without the name) to take prudent measures to prevent the spread of the disease and to cleanse the town from infection. Further acts in 1760 and 1764<sup>78</sup> forbade inoculation entirely and placed the administration of the law in the hands of the same officers. Smallpox was not a quarantinable disease between 1784 and 1827.<sup>79</sup>

Powers for the removal and regulation of nuisances caused by mill dams and stagnant water were given in special acts constituting the county commissioners of certain counties a board of health and drainage,<sup>80</sup> but other local boards of health do not seem to have been usual in South Carolina until after the establishment of the state board of health.

In 1878 South Carolina inaugurated a new and centralized system of control over matters relating to public health. By a law passed in this year<sup>81</sup> a state board was created. The board was composed of the members of the state medical association and was to act through an executive committee consisting of seven members recommended

<sup>73</sup> S. C. St. 1796, ch. 1646; 1797, ch. 1672; 1809, ch. 1944; 1827, ch. 2409; 1832, ch. 2583; 1861, ch. 4528; 1865, ch. 4742.

<sup>74</sup> S. C. St. 1868, ch. 60.

<sup>75</sup> S. C. St. 1869, ch. 132.

<sup>76</sup> S. C. St. 1872, ch. 77.

<sup>77</sup> S. C. St. 1738, ch. 651.

<sup>78</sup> S. C. St. 1760, ch. 892; 1764, ch. 930.

<sup>79</sup> S. C. St. 1784, ch. 1219; 1827, ch. 2409.

<sup>80</sup> S. C. St. 1874, ch. 528.

<sup>81</sup> S. C. St. 1878, ch. 610.

by the association and appointed by the governor, together with the state's attorney and the comptroller general, *ex officio*. The state board was to divide the state into districts, to appoint local boards in such districts as already had none, and to exercise supervisory control over all local boards. It was also given the supervision and control of the quarantine system of the state and was authorized, with the advice and consent of the governor, to establish and maintain quarantine both by land and by sea. Investigations were to be conducted by both state and local boards into the causes of disease and the influence of climate, occupations, and other conditions upon health. An amendment to the quarantine law in 1881<sup>82</sup> provided that quarantine for Charleston should be administered by the city board of health subject to the advice and supervision of the executive committee of the state board, and that the health officer of the port should be appointed by the governor upon nomination of the city board; quarantine at other ports of the state was to remain under the control of the state board, with the immediate execution of the law by a health officer or local board appointed by the executive committee of the state board. After 1883<sup>83</sup> local boards, to be appointed by the mayor and council, were required for all cities and towns; after 1885<sup>84</sup> county boards might be appointed from time to time as necessary by the state board for counties outside of cities and towns. Local boards organized under these two acts were given wide powers over nuisances and other matters affecting the health of the locality and were to maintain and enforce quarantine when declared by the state board and approved by the governor. In case the local authorities failed to appoint a board of health within 60 days such boards were to be appointed by the state board. The supervisory power of the state board over local boards was extended in 1901<sup>85</sup> to the removal of members of such boards upon refusal or neglect to obey orders of the state board. By law of 1895<sup>86</sup> local boards were empowered to require and enforce vaccination, and in 1899<sup>87</sup> a law was passed empowering the state board to enforce vaccination and

<sup>82</sup> S. C. St. 1881, ch. 474.

<sup>83</sup> S. C. St. 1883, ch. 170; from 1895 to 1897 local boards were elected, S. C. St. 1895, ch. 550; 1897, ch. 285.

<sup>84</sup> S. C. St. 1885, ch. 172.

<sup>86</sup> S. C. St. 1895, ch. 550.

<sup>85</sup> S. C. St. 1901, ch. 420.

<sup>87</sup> S. C. St. 1899, ch. 78.

punish by fine all who refused to submit. In addition to the supervisory power of the state board over the local board, a law in 1889<sup>88</sup> authorized the governor, upon recommendation of the chairman of the state board, to appoint sanitary inspectors, who, under the direction of the state board in time of threatened danger from epidemic disease, were to inspect railroad cars, and had the power to stop and detain all passengers, baggage, and freight, and, if necessary to prevent the spread of disease, to disinfect or destroy cars, vessels, baggage, or freight.

Since the first establishment of the state board in 1878 South Carolina has been among the foremost of the states to have a centralized system of control over matters relating to the public health; her method of selecting the board to which the oversight of this highly centralized system is intrusted is unusual.

#### FEDERAL LEGISLATION.

Since the control exercised by the federal government over matters relating to health is incidental to the power over international relations and commerce it is limited in scope and has been directed chiefly toward laying restrictions upon the free movements of men and animals afflicted with disease and toward the inspection of food products intended for interstate and foreign commerce. Federal activity along both these lines has been most jealously regarded by state authorities and no bill upon either subject has become law without bitter controversy. It is not necessary to give here more than a brief summary of federal legislation in regard to quarantine, and to the short-lived National Board of Health.<sup>89</sup>

The federal Congress, in even a more marked degree than the state legislatures, has waited for the sharp prod of a devastating epidemic before taking action in regard to health matters. As early as 1790, during the first session of the newly established Congress, a committee of five was appointed "to bring in a bill to establish health officers in the principal ports of the Union,"<sup>90</sup> but no law was enacted until 1796 after "the late sickness in New York." A resolution was introduced into the House of Representatives<sup>91</sup> by a

<sup>88</sup> S. C. St. 1889, ch. 226.

<sup>89</sup> For a fuller review of federal legislation in regard to health, see an article by J. W. Garner entitled "Federal Activity in the Interest of Public Health" in the *Yale Review*, August, 1905.

<sup>90</sup> Annals of Congress, 1, p. 1817.

<sup>91</sup> *Ibid.*, 5, p. 1228.

member from Maryland that: "the President of the United States be authorized to direct such quarantine to be performed on all vessels from foreign countries arriving at ports of the United States as he shall judge necessary." The resolution was referred to the proper committee and a bill reported embodying the substance of the resolution, but was so amended that, as finally passed,<sup>92</sup> it merely authorized the President to direct certain federal officers to assist the states in carrying into effect their several laws until Congress should make regulations to the contrary. In the debates on this first bill all the arguments were brought forward that have been urged in favor of or against later bills. The enemies of the bill opposed it on the ground that the President was too far from the threatened ports; that too great power would therefore be placed in the hands of the collector of the port; that regulations for the care of public health were not an executive function, but legislative; that the matter would better be left to the states as it was only of local concern; that the bill was unnecessary, as quarantine laws were already observed in some of the states and every state was justified in excluding disease; that quarantine was not at all a question of commerce, but of health, "pestilential disorders are not articles of commerce," and therefore was not within the scope of federal authority; and that, even if the commerce clause of the constitution gave any power in the matter to the federal government, it was better to leave the matter to the states to avoid conflicts of authority and the unnecessary duplication of buildings and officers. The friends of the bill urged on the contrary that quarantine was a part of commerce and navigation and that the states therefore had no power over it.

Two years later, 1798, after another widespread epidemic of yellow fever,<sup>93</sup> the attention of Congress was again called to the necessity for action, this time by President Adams in his annual message:<sup>94</sup> "And when we consider the magnitude of the evils arising from the interruption of public and private business whereby the national interests are deeply affected, I think it my duty to invite the Legislature of the Union to examine the expediency of establishing suitable regulations in aid of the health laws of the respective states; for these being formed on the idea that contagious sickness may be

<sup>92</sup> 1 Stat. at Large, p. 474.

<sup>93</sup> *Supra*, pp. 8, 46.

<sup>94</sup> Annals of Cong., 9, p. 2420.



communicated through the channels of commerce, there seems to be a necessity that Congress, who alone can regulate trade, should frame a system which, while it may tend to preserve the general health, may be compatible with the interests of commerce and the safety of the revenue." Following the lines indicated in the message a bill was introduced and passed in February, 1799, without recorded debate.<sup>95</sup> It provided for the observance of state laws regarding quarantine by various classes of federal officers, for the discharge and deposit of goods from infected ships in suitable warehouses, and for the removal of federal offices to places of safety in time of epidemic. Such as it was this law remained on the statute books as the basis of federal quarantine laws for nearly 80 years. Memorials begging for general quarantine laws came up from the board of health and common council of Philadelphia in 1800;<sup>96</sup> in 1802 the merchants of New York followed,<sup>97</sup> inveighing against the inconvenience of the state laws; but these petitions from the two most important seaports had no visible effect upon Congress; there was no further quarantine legislation of any kind until the year of the first invasion of Asiatic cholera, 1832. At that time a bill was introduced into the House<sup>98</sup> to appropriate \$50,000 to be expended under the authority of the President "in endeavoring to preserve the people of this country from the prevalence of the disease known by the name of Asiatic cholera, now raging in Canada," but the appropriation was cut out and the act as passed merely permitted the secretary of the treasury to assist the states in enforcing their quarantines.<sup>99</sup> In 1866 at the time of another invasion of cholera a similar resolution to that of 1832 met a better fate<sup>100</sup> and money was appropriated to aid the states in their fight against cholera. In 1869 a resolution was introduced<sup>101</sup> requesting the secretary of the treasury to report as to the feasibility of adopting a uniform system of quarantine laws, the execution to be under the Treasury Department, but this suggestion met with no encouraging response, nor did a resolution introduced in 1871 by a member from Texas for a more effective system of quarantine on the gulf coast.<sup>102</sup>

<sup>95</sup> I Stat. at Large, p. 619.

<sup>96</sup> Annals of Cong., 10, pp. 63, 578, 112, 633.

<sup>97</sup> *Ibid.*, 11, p. 991.

<sup>98</sup> Congressional Debates, 8, p. 3677.

<sup>99</sup> IV Stat. at Large, p. 577.

<sup>100</sup> XIV Stat. at Large, p. 357.

<sup>101</sup> Congress. Globe, 40th Cong., Part I, p. 415.

<sup>102</sup> *Ibid.*, 41st Cong., 3d sess., Part II, p. 1438.



At about this time, 1871-73, several attempts were made to direct the attention of the national legislators to the necessity for federal action to prevent the introduction of contagious disease, particularly the constant invasions of yellow fever, but the only one which brought about any action was that of June 6, 1872, directing the secretary of war to detail medical officers from the regular army to visit ports subject to yellow fever, to confer with the local authorities, and ascertain facts as to previous outbreaks and possible means of prevention.<sup>103</sup> The reports submitted in accordance with this resolution through the surgeon-general became the basis of several bills introduced into the next Congress.<sup>104</sup> The early bills were lost in committee, but a terrible epidemic of yellow fever in the summer of 1873 aroused the public, and numerous memorials from southern cities prayed for national action. It is interesting to note that all the later legislation on the subject of national control of health matters has been introduced by members from those states which have been noted for their adherence to the theory of states' rights. "The people of the entire gulf coast are united in requesting the interposition of national power to protect them, and through them the entire southwest, from the terrible visitations of imported plagues. . . . The people of the South are not so in love with national power that they would resort to it as a cure for any evil they may labor under had not sad experience shown not only the necessity of such appeal, but also demonstrated why the remedy lies in the national power alone. . . . A sanitary line is like a military one, as strong only as its weakest point." A bill was introduced at this time by an Alabama member,<sup>105</sup> providing for a national board of health to consist of three: the surgeons-general of the army, of the navy, and of the Marine Hospital, who should have power, subject to the approval of the President, to prescribe the times, manner, and place of performing quarantine for all vessels and vehicles entering the United States from a foreign port or place, and to make all needful rules and regulations for the efficient execution of the purposes of the law. It also gave to the President power to detail officers from the medical departments of the army, navy, or Marine Hospital to carry out such regulations. But the bill,

<sup>103</sup> XVII Stat. at Large, p. 396.

<sup>105</sup> *Ibid.*, 2, Part III, pp. 2887, 2998, 4611.

<sup>104</sup> Congress. Record, 2, pp. 1003, 2998.

although passed in a modified form by the House, was lost in the Senate.

There was more or less yellow fever along the southern coasts during the next two or three summers, somewhat more in 1877. A memorial from a convention of southern cities in February of 1878 prayed for a uniform and effective system of national quarantine as the only reliable means of protection against disease from foreign countries. Southern members introduced a bill,<sup>106</sup> urged its immediate passage, and granted that unlimited authority to exclude this "imported disease" was vested in Congress by the commerce clause; but northern advocates of state power were found to oppose jealously the giving of more authority to the federal government and amendments were forced upon the bill until, as passed,<sup>107</sup> it was utterly useless and "altogether subservient to the state regulations." The only clause of value was that directing consuls to give weekly reports of the sanitary condition of foreign ports and to notify the surgeon-general and local health officers of vessels bound for the United States which left infected ports or had infected persons on board.

Scarcely was the ink of the President's signature dry on the new law when the grim scourge came to mock the futility of such measures. The summer of 1878 saw the worst epidemic of yellow fever known to our history. In his message to Congress in December of that year<sup>108</sup> President Hayes called attention once more to the necessity for federal action: "The fearful spread of this pestilence has awakened a very general public sentiment in favor of national sanitary administration, which shall not only control quarantine, but have the sanitary supervision of internal commerce in times of epidemics and hold an advisory relation to the state and municipal authorities with power to deal with whatever endangers the public health and which the municipal and state authorities are unable to regulate." On the first day of the session, even before the President's message had been received, three resolutions calling for federal action were presented by senators from three southern states. A bill was presented<sup>109</sup> for the creation of a bureau of health in the Treasury Department with adequate power to enforce its own regulations in case of

<sup>106</sup> *Ibid.*, 7, pp. 1603, 2074.

<sup>107</sup> XX Stat. at Large, p. 37.

<sup>108</sup> Congress. Record, 8, p. 3.

<sup>109</sup> *Ibid.*, 8, p. 2.

the failure of local officers to do so; this bill was vigorously opposed by northern senators on constitutional grounds, but passed the Senate only to fail in the House. In spite, however, of the opposition of members of Congress from New York, Massachusetts, and other states having good quarantine systems, the question of federal regulation of matters affecting public health was not allowed to drop, and an act was passed on March 3, 1879, to create a national board of health.<sup>110</sup> The national board as thus created consisted of seven representative physicians appointed by the President from as many different states, together with three medical officers detailed from the army, the navy, and the Marine Hospital Service, and one officer from the Department of Justice; it had no power or authority over quarantine, but was a purely investigative and advisory body.

On June 2 of the same year a further act was passed<sup>111</sup> which gave to the board its first substantial power. By its terms all vessels arriving at any port of the United States were required to present a bill of health obtained from the consular or medical representative of the United States at the port of departure. The power to make rules and regulations to be observed by vessels at the port of departure and during the voyage was transferred from the surgeon-general of the Marine Hospital Service to the National Board of Health, as was also the duty to publish weekly abstracts of the sanitary reports required of consuls. The board was also empowered to aid state and municipal boards of health in the execution of their quarantine laws and to make quarantine regulations at the request of the President where, in his opinion, local regulations were inadequate; upon the refusal or failure of the state authorities to execute such regulations they were to be executed by an officer detailed or appointed for the purpose. An appropriation of \$500,000 was made to aid the state authorities in enforcing their quarantine laws. Further power was granted to the board by the act of July 1, 1879,<sup>112</sup> to erect temporary quarantine buildings.

Almost immediately after the establishment of the National Board of Health there began to be manifest a strong feeling of jealousy and hostility toward it, not only by local boards, especially at New York and New Orleans, but also by the Marine Hospital Service; no stone

<sup>110</sup> XX Stat. at Large, p. 484.

<sup>111</sup> XXI Stat. at Large, p. 5.

<sup>112</sup> XXI Stat. at Large, p. 46.

was left unturned to bring the national board into disrepute. There were many attempts to increase the efficiency of the board but all such attempts were met by determined and bitter opposition on the part of the members of Congress from New York and Louisiana, and the dependence of the board upon the yearly appropriations of Congress for its support furnished a most effective weapon to its enemies. These appropriations dwindled and finally ceased. In 1882<sup>113</sup> the functions of the board were limited to the investigation of the three diseases of cholera, smallpox, and yellow fever; control over quarantine stations was transferred to the Marine Hospital Service in 1888,<sup>114</sup> and finally, in February, 1893,<sup>115</sup> a transfer of property was made to the Marine Hospital Service and the law creating the board was repealed.

Scarcely a session of Congress has passed since 1879 without petitions for the creation of a national board with adequate powers over matters relating to health, especially foreign and interstate quarantine; many bills have been introduced<sup>116</sup> for a federal quarantine, but the opposition of the Marine Hospital Service was strong enough to prevent their passage until after the control over such quarantine as existed had become firmly established in their own hands.<sup>117</sup> In 1888 immediately after the transfer of the quarantine stations from the National Board of Health to the Marine Hospital Service eight new stations were established.<sup>118</sup>

In 1890<sup>119</sup> a law was passed authorizing the President, in time of threatened danger from cholera, smallpox, yellow fever, or plague, to cause rules and regulations to be made by the surgeon-general of the Marine Hospital Service and promulgated through the secretary of the treasury to prevent the spread of these diseases from one state or territory to another; the President was also authorized to employ such persons as were necessary to execute these regulations and to prevent the spread of such diseases. For the first time we hear nothing of aid to be rendered to the states in support of state quarantine regulations. In the following year, 1891, a law was passed<sup>120</sup> for the exclusion

<sup>113</sup> XXII Stat. at Large, p. 375.

<sup>115</sup> XXVII Stat. at Large, p. 449.

<sup>114</sup> XXV Stat. at Large, p. 355.

<sup>116</sup> Congress. Record, 16, p. 2872; 20, p. 1455, etc.

<sup>117</sup> "While the National Board of Health existed it was crippled, embarrassed, and finally starved to death by the active hostility of the Marine Hospital Service." (Speech of Senator Harris of Tennessee, Congress. Record, 24, p. 395.)

<sup>118</sup> XXV Stat. at Large, p. 355.

<sup>120</sup> XXVI Stat. at Large, p. 1084, sec. 8.

<sup>119</sup> XXVI Stat. at Large, p. 31.



of immigrants suffering from contagious or loathsome diseases with provision for a thorough medical examination of all suspected cases; the duties and powers of state officers in regard to the inspection of immigrants were transferred to federal officers appointed for that purpose.

In the summer of 1892 cholera appeared at various places in Europe; it was especially prevalent at the port of Hamburg, and was brought thence by steamers to the harbor of New York City; panic ensued and there followed a clash of authority between the federal officers and the quarantine officers of the state of New York; the latter were accused of inefficiency and the New York Chamber of Commerce petitioned Congress for the establishment of a national system of quarantine. A number of bills were introduced and in February, 1893,<sup>121</sup> an act was passed which was the first national quarantine law with any semblance of power. Under this law whenever the secretary of the treasury considers the rules and regulations of any state inadequate to prevent the introduction of contagious disease from foreign countries or from one state to another he is empowered to make and promulgate such additional rules and regulations as may, in his opinion, be necessary. These are to be enforced by state or municipal authorities but if they refuse or neglect to enforce them the President is empowered to detail or appoint officers for that purpose. No vessel may enter any port of the United States without a bill of health, nor without a certificate of inspection performed according to federal regulations. In case of the arrival of an infected vessel at any port not provided with proper facilities for disinfection the secretary of the treasury may remand the vessel to any national or other quarantine station provided with accommodations and appliances for the treatment and disinfection of passengers, cargo, and vessel; a certificate from the United States officer at such a station that the vessel, passengers, and cargo are free from infectious disease admits without further question to the port of entry named in the certificate. The act further provides for suitable compensation to be paid to the authorities of any state upon the surrender to the United States of the use of the state quarantine stations or disinfecting apparatus necessary to the United States. It also places in

<sup>121</sup> XXVII Stat. at Large, p. 449.



the hands of the President the power to suspend immigration entirely in times of danger from the introduction of contagious disease. Even this law, although marking a great advance, failed to place entire control of quarantine under the federal government, since the surgeon-general of the Marine Hospital, the executive officer of the federal system, is required to "co-operate with and aid state and municipal boards of health in the execution and enforcement" of their rules and regulations, and it is only when such rules and regulations are inadequate that they are supplemented or superseded by federal regulations.

This law of 1893 is the basis of the present federal law upon the subject of quarantine. Several bills were introduced<sup>122</sup> in 1899 to increase the federal power: to maintain a system of quarantine, to establish a bureau of health in the Treasury Department, and to establish a national board of health, but all failed to become law.

In 1900 the quarantine regulations were extended to Porto Rico and Hawaii<sup>123</sup> and in 1903 to vessels entering the United States or territories from the Philippine Islands.<sup>124</sup>

In 1901 the law of 1893 was amended<sup>125</sup> by increasing the penalties imposed upon masters of vessels transgressing the quarantine regulations, by subjecting vessels approaching any port of the United States without a bill of health to such regulations as the secretary of the treasury might impose, and by authorizing medical officers of the United States to administer oaths. The provisions of the Alien Immigration Act were also made more strict with regard to the deportation of diseased aliens in 1903.<sup>126</sup>

Two other acts passed within the past six years have tended greatly toward establishing a truly national system of quarantine. By the first of these laws, that of July 1, 1902,<sup>127</sup> the name of the Marine Hospital Service was changed to "The Public Health and Marine Hospital Service of the United States." An advisory board was created for consultation with the surgeon-general in regard to the investigations to be undertaken by the Hygienic Laboratory; this board was to consist of three competent experts detailed from

<sup>122</sup> Congress. Record, 31, pp. 57, 223, 887, 971.

<sup>124</sup> XXXII Stat. at Large, p. 691.

<sup>123</sup> XXXI Stat. at Large, pp. 80, 160.

<sup>125</sup> XXXI Stat. at Large, p. 1086.

<sup>126</sup> XXXII Stat. at Large, p. 1213, SECS. 2, 9, 10, 12, 14, 17.

<sup>127</sup> XXXII Stat. at Large, p. 712.

the army, the navy, and the Bureau of Animal Industry, together with the director of the laboratory and five other members, not in the employ of the government, to be appointed by the surgeon-general with the approval of the secretary of the treasury. The board so established was to hold one or more conferences not to exceed 10 days per annum. The surgeon-general was also authorized to call an annual conference of state and territorial boards of health, quarantine authorities, and state health officers, one delegate from each state or territory, and such other conferences as, in his opinion, would promote the public health; it was also made the duty of the surgeon-general to call a conference upon application of five state or territorial officers or boards. An attempt was made to secure uniformity of registration of vital statistics by a provision for reports upon blanks provided for the purpose by the surgeon-general.

By the law of June 19, 1906,<sup>128</sup> the secretary of the treasury was given control, direction, and management of all quarantine stations, he was to select as soon as practicable four additional sites, best suited to prevent the introduction and spread of yellow fever, and to establish a quarantine and disinfection station at the Dry Tortugas to which he might send any vessel, bound for any port of the United States, for treatment and disinfection until all danger of contagion was removed. After the publication of the sites of these stations and of the regulations with regard to them, vessels infected with yellow fever were required to go directly to them without entering any port of the United States. Before establishing stations at other points the secretary of the treasury was required to examine the stations already provided by the local authorities, and, if suitable for the purpose, to negotiate with the local authorities for their purchase; but the purchase could be completed only after jurisdiction over the site in question had been ceded by the state to the United States government.

The only other act of importance to health in recent years was an act of March 3, 1905, providing for a special investigation of leprosy in Hawaii.<sup>129</sup>

It is always interesting to test the growth of legislative interest in any subject by a comparison of the appropriations. In 1879, the

<sup>128</sup> XXXIV Stat. at Large, p. 299.

<sup>129</sup> XXXIII Stat. at Large, p. 1009.

year of the establishment of the National Board of Health, \$50,000 was appropriated for the salaries and all other necessary expenses of the board, also \$5,000 for the use of the Marine Hospital Service in preparing bulletins of health; in 1906 \$340,000 was appropriated for the support of 30 or more quarantine stations, \$200,000 as an emergency fund at the disposal of the President for the suppression of epidemics, and \$1,226,380 for salaries and general expenses of the Public Health and Marine Hospital Service, in addition to an appropriation for the leper hospital in Hawaii, a total of \$1,566,380.

## APPENDIX B.

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## APPENDIX D.

### TABLE OF EPIDEMICS AND RESULTING LEGISLATION.

The following table is intended to show the great epidemics which have visited the principal cities of the United States with the ensuing legislation. The figures are necessarily incomplete, especially before 1800; statistics of mortality were not carefully kept until recent years. Epidemics of smallpox were of such common occurrence that few records of them are obtainable; we read, however, that such epidemics occurred in New York, Philadelphia, and Charleston "during nearly every decade of the seventeenth and eighteenth centuries." Where estimates have varied widely the lowest and highest figures have been given. In several cases statistics of great epidemics in foreign countries have been given, but the table is so arranged that it can be seen at a glance whether foreign cities or those of the United States were affected; the names of the foreign cities being placed to the right of the column. The names of the three diseases which have produced our great epidemics have also been placed in such a way that each can be easily followed from year to year. It should be borne in mind that a law enacted in consequence of an epidemic frequently bears the date of the following year; there might have been no session of the legislature for several months after the cessation of the epidemic. It is interesting also to notice the character of the legislation following epidemics; in general quarantine laws have followed yellow fever; laws against nuisances, cholera.

Year	Place	Estimated Population	Epidemic Disease	No. Cases (or Character)	No. Deaths	Subject of Resulting Law (Order, Ordinance)
1647	New Netherlands	1,000	Yellow Fever	.....	6,000-12,000	1647, Mass. Quarantine (Orders)
1663		.....	Smallpox	most malignant	.....	N. Y. Quarantine (Orders)
1665		.....	.....	.....	.....	N. Y. City (Ordinances)
1668		.....	.....	.....	.....	Nuisances
	All New England Massachusetts		Gt. Plague			1665, Mass. Quarantine (Orders)
			Yellow Fever (?)	"The year of the great sickness,"		
1672		8,000	Yellow Fever (?)	very fatal		
1677		.....	Yellow Fever (?)	.....		
1678	Massachusetts	.....	Smallpox	.....	700-800	
		.....	Smallpox	.....		
1680-90		.....	Smallpox	.....		
1691		.....	Yellow Fever (Siam Distemper)	.....	$\frac{1}{3}$ of population	
1693	Boston	.....	"Barbadoes Distemper," (Yellow Fever)	.....	.....	1693, S. C. Quarantine (by "Resolution of Inhabitants")
	Charleston, S. C.	.....	Yellow Fever	.....	.....	1698, S. C. Quarantine
1699	Philadelphia	3,800	"Barbadoes Distemper," (Yellow Fever)	.....	220	1699, N. Y. (Ordinance) Public Scavenger
	Charleston	.....	Yellow Fever	.....	.....	1700, Pa. Quarantine
1700	Charleston	.....	Smallpox	fatal	.....	Mass. Quarantine
1701	Philadelphia	.....	Smallpox	"mortal and general"	.....	1701, Mass. Quarantine
1702	Boston	.....	Smallpox	.....	.....	Mass. Quarantine by Towns
	New York	6-7,000	Yellow Fever	"the great sickness"	570-800	1702, N. Y. Quarantine (Orders)
1703	Charleston	.....	Yellow Fever	.....	.....	N. Y. (Ordinance) St. Cleaning
1705	Cadiz, Spain	.....	Yellow Fever	.....	.....	1704, S. C. Nuisances
1710	Philadelphia	.....	Smallpox	.....	.....	1712, S. C. Quarantine
1717	Charleston	.....	Yellow Fever	.....	.....	1717-18, Mass. Quarantine
	France	.....	Smallpox	.....	.....	1721-2, Mass. Quarantine
1721	Charleston	.....	Smallpox	.....	.....	S. C. Quarantine
1728	Charleston	.....	Yellow Fever	.....	.....	
1730	Baltimore	13,000	Smallpox	4,000	500	
1730-31	Philadelphia	.....	Smallpox	288	500	
	New York	.....	Smallpox	.....	.....	
1732	Charleston	.....	Smallpox	lasted 90 days	10-12 daily	
	Charleston	.....	Yellow Fever	.....	70	
1734	New York	.....	Yellow Fever	.....	.....	
	Charleston	.....	Yellow Fever	.....	.....	



Year	Place	Estimated Population	Epidemic Disease	No. Cases (or Character)	No. Deaths	Subject of Resulting Law, (Order, Ordinance)
1738.....	Charleston	....	Smallpox	2,112	411	1738, S. C. Inoculation, Isolation, etc.
1739.....	New York	....	Smallpox	.....	.....	Mass. Quarantine
1741.....	Charleston	....	Yellow Fever	very fatal	.....	1739, Mass. Quarantine by Land
	Philadelphia	....	Yellow Fever	.....	250-595	
	New York	....	Yellow Fever	.....	.....	
1742.....	Spain	....	Yellow Fever	.....	10,000	
	Philadelphia	....	Yellow Fever	.....	.....	1742, Pa. Quarantine, Hospital
1743.....	New York	....	Yellow Fever	.....	.....	
1745.....	New York	7-8,000	Yellow Fever	.....	217-279	1744, S. C. Quarantine
	Charleston	....	Yellow Fever	mild	.....	
1746.....	New York	....	Yellow Fever	.....	.....	
	New York	....	Smallpox	.....	.....	
1747.....	Albany	....	Yellow Fever	.....	45	1747, S. C. Quarantine
	Philadelphia	....	Yellow Fever	light	.....	
1748.....	New York	....	Yellow Fever	.....	.....	
1751.....	Charleston	....	Yellow Fever	fatal	.....	1749, S. C. Quarantine
1752.....	New York	....	Yellow Fever	.....	.....	1751, Mass. Quarantine
1754.....	Boston	15,700	Smallpox	7,669	569	1752, S. C. Quarantine
1755.....	Philadelphia	....	"Dutch Distemper" (?)	.....	.....	1755, N. Y. First Quarantine Law
1756.....	Philadelphia	....	Smallpox	.....	.....	1756, S. C. Quarantine
1760.....	Charleston	....	Smallpox	.....	940	1759, S. C. Inoculation
1762.....	New York	....	Yellow Fever	.....	20 each day	1762, Pa. Sewage
1763.....	Philadelphia	....	Yellow Fever	.....	259	1763, Mass. Inoculation
	Nantucket Island	....	Yellow Fever	.....	.....	N. Y. Inoculation
1764.....	Charleston	6,000	Smallpox	.....	.....	Mass. Inoculation
1764.....	Boston	15,500	Smallpox	5,646	170	S. C. Inoculation
1769.....	New Orleans	....	Yellow Fever	.....	.....	
1773-4.....	Philadelphia	....	Smallpox	.....	.....	1774, Pa. Quarantine
	Boston	....	Smallpox	.....	.....	1775, Mass. Local Quarantine vs. Smallpox
1776.....	Philadelphia	....	Smallpox	5,202	57	1776, Mass. Inoculation
1778.....	Boston	13,500	Smallpox	2,243	61	1783, S. C. Quarantine
1780.....	Charleston	....	Smallpox	.....	.....	1784, S. C. Quarantine
1791.....	New York	33,131	Yellow Fever	.....	200	1784, N. Y. Quarantine
1792.....	New Orleans	16,484	Yellow Fever	.....	198	1792, Mass. Inoculation
1793.....	Boston	....	Smallpox	8,346	.....	Mass. Quarantine Resol.
	Charleston	30-50,000	Yellow Fever	.....	4041	Pa. Quarantine Resol.
	Philadelphia	....	Yellow Fever	.....	.....	

Year	Place	Estimated Population	Epidemic Disease	No. Cases (or Character)	No. Deaths	Subject of Resulting Law, (Order, Ordinance)
1794.....	Philadelphia New York Charleston	..... ..... .....	Yellow Fever Yellow Fever Yellow Fever	..... 20-30 .....	70-100 ..... .....	1794, Pa. Quarantine N. Y. Quarantine
1795.....	New York Philadelphia Charleston Norfolk, Va.	58,000 ..... ..... .....	Yellow Fever Yellow Fever Yellow Fever Yellow Fever	..... 2,404 ..... 220	525-750 ..... ..... .....	1795, Pa. Quarantine
1796.....	New York Philadelphia Boston Charleston Gallipolis, Ohio	..... ..... ..... ..... .....	Yellow Fever Yellow Fever Yellow Fever Yellow Fever Yellow Fever	..... ..... ..... ..... .....	70 ..... ..... ..... .....	1796, N. Y. Quarantine, Noxious Trades 1796, U. S.
1797.....	New York Philadelphia Charleston Baltimore Providence	..... 65,000 ..... ..... .....	Yellow Fever Yellow Fever Yellow Fever Yellow Fever Yellow Fever	..... ..... ..... ..... .....	23 1,000-1,202 ..... 545 45	1797, N. Y. Quarantine, Nuisances 1797, Mass. Quarantine, Town Boards Health 1797, S. C. Quarantine
1798.....	Portsmouth, N. H. Boston New York Philadelphia Wilmington, Del. Baltimore	..... ..... 50,000 ..... ..... .....	Yellow Fever Yellow Fever Yellow Fever Yellow Fever Yellow Fever Yellow Fever	..... ..... ..... ..... ..... .....	100 200-300 1,500-2,700 3,521-3,645 250 200	1798, N. Y. Quarantine, Nuisances Mass. Nuisances
1799.....	New York Philadelphia Charleston Norfolk, Va.	66,000 ..... 41,220 18,824	Yellow Fever Yellow Fever Yellow Fever Yellow Fever	..... ..... ..... .....	70 1,000-1,276 230 .....	1799, Mass. Quarantine, Nuisances 1799, N. Y. Quarantine, Nuisances 1799, Pa. Quarantine, Nuisances 1799, U. S. Quarantine
1800.....	.....	.....	.....	.....	.....	1800, N. Y. Quarantine
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Year	Place	Estimated Population	Epidemic Disease	No. Cases (or Character)	No. Deaths	Subject of Resulting Law, (Order, Ordinance)
1804	New York Charleston	65,500	Yellow Fever	.....	708	1805, N. Y. Nuisances
		.....	Yellow Fever	.....	148	
1805	Spain	.....	Yellow Fever	.....	124,000	
	New York Philadelphia	75,770	Yellow Fever	.....	270-340	1806, N. Y. Quarantine, Nuisances
		.....	Yellow Fever	600-645	300-400	1806, Pa. Quarantine, Nuisances
1806	Quebec	.....	Yellow Fever	.....	.....	1806, S. C. Quarantine
1807	New York	.....	Yellow Fever	.....	.....	
1807	Charleston	.....	Yellow Fever	.....	162-176	1809, S. C. Quarantine by armed force
1807	Brooklyn	.....	Yellow Fever	.....	30-40	1811, N. Y. Quarantine
1813	Charleston	130,000	Yellow Fever	.....	4,000	1811, Pa. Quarantine
1817	New Orleans	.....	Yellow Fever	.....	270	1817, Pa. Quarantine
	Boston	.....	Yellow Fever	.....	800	1818, Pa. Quarantine, Nuisances
1819	New York	.....	Yellow Fever	.....	34	
	Charleston	42,293	Yellow Fever	63-150	38-50	1820, N. Y. Quarantine
	New Orleans	.....	Yellow Fever	.....	176	
	Havana	.....	Yellow Fever	.....	2,160	
	Cadiz	.....	Yellow Fever	.....	5,162	
1820	New York	72,000	Yellow Fever	.....	5,000-8,000	
	Philadelphia	123,796	Yellow Fever	.....	150	
	New Orleans	63,862	Yellow Fever	.....	67-84	1821, Pa. Nuisances
1821	New York	.....	Yellow Fever	103	400	
	Barcelona	125,000	Yellow Fever	.....	132	
1822	New York	145,000	Yellow Fever	140	9,730-25,000	1823, N. Y. Quarantine
	New Orleans	124,000	Yellow Fever	401-422	230-300	
1823	New York	28,000	Yellow Fever	.....	239-800	1824, Pa. Inoculation
	Charleston	.....	Yellow Fever	.....	.....	
1824	New Orleans	27,822	Yellow Fever	.....	28-235	
	Gibraltar	.....	Yellow Fever	.....	108	
1825	Charleston	20,652	Yellow Fever	6,715	1,790	
1826	Charleston	28,233	Smallpox	.....	52	
1827	Charleston	28,644	Smallpox	.....	29	1827, S. C. Quarantine vs. Smallpox
	New Orleans	29,955	Yellow Fever	.....	64	
1828	Charleston	.....	Yellow Fever	.....	100	
	New Orleans	29,466	Yellow Fever	.....	26	
1829	New Orleans	.....	Yellow Fever	.....	130	
1830	New Orleans	.....	Yellow Fever	.....	215	
	Charleston	46,682	Yellow Fever	.....	117	
1831-2	Paris	.....	Yellow Fever	.....	32	
	London	800,000	Cholera	.....	20,000	
	Berlin	1,500,000	Cholera	.....	1,223-5,274	
	Vienna	340,000	Cholera	11,020	1,401	
	St. Petersburg	300,000	Cholera	.....	11,896	
	Moscow	300,000	Cholera	.....	4,757	
		350,000	Cholera	.....	4,600	

Year	Place	Estimated Population	Epidemic Disease	No. Cases (or Character)	No. Deaths	Subject of Resulting Law (Order, Ordinance)
1832.....	Quebec	22,000	Cholera	11,790	2,218-3,513	1832, N. Y. Quarantine for State, Nuisances
	Montreal	25,000	Cholera	4,835	1,843-1,904	Pa. Quarantine
	Boston	202,000	Cholera	5,835	2,251-3,500	S. C. Quarantine
	Philadelphia	150,000	Cholera	3,197	638-935	U. S. Aid to State Quarantine
1833.....	New Orleans	55,084	Cholera	.....	4,340-4,740	
1833.....	New Orleans	.....	Yellow Fever	.....	210-400	
1833.....	New Orleans	.....	Yellow Fever	.....	1,000	
1833.....	New Orleans	.....	Cholera	.....	1,000	
1834.....	Havana	100,000	Cholera	.....	10,000	
1834.....	New York	250,000	Cholera	.....	971	
1835.....	Charleston	29,879	Yellow Fever	.....	49	1836, N. Y. Quarantine
1835.....	New Orleans	29,879	Yellow Fever	.....	284	
1835.....	Charleston	29,879	Yellow Fever	.....	25	
1836.....	Charleston	29,673	Yellow Fever	.....	25	
1837.....	Charleston	.....	Yellow Fever	.....	302	
1837.....	New Orleans	.....	Yellow Fever	.....	442-1,300	1838, Pa. Quarantine
1838.....	Charleston	29,467	Yellow Fever	.....	354	
1839.....	Charleston	29,304	Yellow Fever	.....	22-133	
1839.....	New Orleans	.....	Yellow Fever	.....	452	
1839.....	Mobile	.....	Yellow Fever	1,000	650	
1839.....	Mobile	.....	Yellow Fever	.....	250	
1840.....	Galveston	.....	Yellow Fever	.....	22	
1840.....	Charleston	29,261	Yellow Fever	.....	594-1,325	
1841.....	New Orleans	.....	Yellow Fever	.....	211	
1842.....	New Orleans	.....	Yellow Fever	.....	487	
1843.....	New Orleans	.....	Yellow Fever	1,000 +	183	
1843.....	New Orleans	.....	Yellow Fever	772	148	1846, N. Y. Quarantine
1844.....	New Orleans	2,757	Yellow Fever	.....	400	
1844.....	Galveston	.....	Yellow Fever	.....	100	
1846.....	New Orleans	.....	Yellow Fever	.....	200	
1847.....	New Orleans	.....	Yellow Fever	.....	850-872	
1847.....	Galveston	.....	Yellow Fever	1,000 +	15,067	1848, Pa. Nuisances
1848.....	New Orleans	.....	Yellow Fever	33,000 +	53,293	Mass. Nuisances
1848-9.....	Paris	.....	Cholera	.....	924	1849, N. Y. Quarantine, Nuisances
1848-9.....	England	.....	Cholera	.....	3,000-5,500	1849, Pa. Nuisances
1849.....	New Orleans	122,000	Cholera	.....	1,012	
1849.....	New York	500,000	Cholera	2,884	611	
1849.....	Philadelphia	120,000	Cholera	950	859	Mass. Nuisances
1849.....	Boston	135,000	Cholera	.....	678	N. Y. Quarantine, Nuisances
1849.....	Buffalo	42,261	Cholera	.....	1,400-4,114	Pa. Quarantine, Nuisances
1849.....	Chicago	.....	Cholera	.....	953-4,555	
1849.....	Cincinnati	.....	Cholera	.....	.....	
1849.....	St. Louis	.....	Cholera	.....	.....	

Year	Place	Estimated Population	Epidemic Disease	No. Cases (or Character)	No. Deaths	Subject of Resulting Law (Order, Ordinance)
1840.....	New Orleans	115,000	{ Yellow Fever Cholera	.....	2,081	
1840.....	Charleston	42,000	Yellow Fever	.....	737	
1850.....	Chicago	29,903	Cholera	.....	125	
1850.....	Cincinnati	115,435	Cholera	.....	420	
	St. Louis	77,860	Cholera	.....	1,004	
	San Francisco	.....	Cholera	.....	883-053	
	Sacramento	8,000	Cholera	.....	250	
	New Orleans	116,375	{ Yellow Fever Cholera	.....	1,000	
1851.....	Chicago	.....	Yellow Fever	.....	1,448	
	St. Louis	.....	Cholera	.....	216	
	New Orleans	.....	Cholera	.....	884	
1852.....	New York	.....	Cholera	.....	430	1852, Pa. Nuisances
	Chicago	.....	Cholera	.....	374	
	St. Louis	.....	Cholera	.....	630	
	New Orleans	.....	Cholera	.....	802	
1853.....	Charleston	42,985	{ Yellow Fever Yellow Fever	.....	1,080-1,320	
	Philadelphia	.....	Yellow Fever	.....	415	
	New Orleans	.....	Yellow Fever	.....	200-310	
	Galveston	.....	Yellow Fever	.....	128	
1853-4.....	New York	100,000	{ Yellow Fever Cholera	170 15,858-20,020	5,613-8,647	
1854.....	Philadelphia	.....	Yellow Fever	.....	554-585	
	Detroit	.....	Yellow Fever	.....	536	
	Chicago	.....	Cholera	.....	12,5725	
	St. Louis	.....	Cholera	.....	2,000-2,810	
	New Orleans	.....	Cholera	.....	500	1855, Pa. Nuisances Mass. Nuisances
	Galveston	.....	Cholera	.....	1,000	
	New Orleans	.....	Cholera	.....	1,454	
	Charleston	.....	Cholera	.....	1,534-3,547	
	Savannah	.....	Cholera	.....	200+	
	Galveston	.....	Cholera	.....	2,423-2,484	
1855.....	New Orleans	.....	{ Yellow Fever Yellow Fever	.....	627	
	Charleston	.....	Yellow Fever	.....	580	
	Savannah	.....	Yellow Fever	.....	404	
	Galveston	.....	Yellow Fever	.....	883	
	New Orleans	.....	{ Yellow Fever Cholera	.....	2,670-3,073	1856, N. Y. Quarantine
	Norfolk	.....	Yellow Fever	.....	2,807	
	Portsmouth	.....	Yellow Fever	.....	500	
1856.....	New York (Staten Is.)	650,000	Yellow Fever	530	211	
1857.....	Charleston	200,000	Yellow Fever	.....	5,652	
	Lisbon	.....	Yellow Fever	13,757	100	
	New Orleans	.....	Yellow Fever	.....	344	
1858.....	Galveston	.....	Yellow Fever	.....	3,889-4,030	
	New Orleans	.....	Yellow Fever	.....	.....	



Year	Place	Estimated Population	Epidemic Disease	No. Cases (or Character)	No. Deaths	Subject of Resulting Law (Order, Ordinance)
1858.....	Galveston	.....	Yellow Fever	.....	344-873	
1859.....	Galveston	7,307	Yellow Fever	.....	717	1863, N. Y. Quarantine
1864.....	Galveston	.....	Yellow Fever	.....	182	1865, N. Y. Quarantine
1866.....	Berlin	.....	Cholera	8,166	259	S. C. 2d. Cholera
	Liverpool	.....	Cholera	.....	6,781	1866, N. Y. Nuisances, Appeal
	New York	.....	Cholera	.....	1,792	Mass. Nuisances, Appeal
	Philadelphia	.....	Cholera	.....	1,212-3,701	U. S. Aid to State Quarantine
	New Orleans	.....	Cholera	.....	809	1867, N. Y. Nuisances
	Chicago	.....	Cholera	.....	1,294	
1867.....	New Orleans	.....	Yellow Fever	.....	900	
	Galveston	.....	Yellow Fever	.....	3,003-3,460	1868, S. C. Quarantine
1870.....	Philadelphia	674,022	Yellow Fever	.....	1,150	1869, S. C. Quarantine
	New Orleans	101,418	Yellow Fever	.....	18	
1871.....	Charleston	48,956	Yellow Fever	.....	587	1872, S. C. Quarantine (also railroads)
1871-2.....	Philadelphia	.....	Small pox	.....	213	U. S. Investigating Board
1873.....	Europe	.....	Cholera	4,464	.....	
	Chicago	208,077	Cholera	.....	.....	
	Cincinnati	216,339	Cholera	.....	.....	1874, N. Y. Nuisances
	St. Louis	310,864	Cholera	593	297-273	Pa. Nuisances (Cities)
	New Orleans	.....	Cholera	.....	392-529	
	Memphis	40,226	Yellow Fever	.....	.....	
1874.....	Charleston	.....	Yellow Fever	343	107-471	
1876.....	Charleston	.....	Yellow Fever	.....	1,244-2,000	
	New Orleans	239,378	Yellow Fever	.....	40	
	Memphis	56,000	Yellow Fever	21,234	30	1878, S. C. State Board, Quarantine
	Vicksburg	14,257	Yellow Fever	.....	4,039	U. S. Consular Reports
	[Whole South]	.....	Yellow Fever	17,600-17,913	4,396-5,150	U. S. National Board, Bills of Health
				5,791	25,000	1879, Pa. Nuisances
				.....	.....	1879, S. C. Quarantine
1892.....	Hamburg	.....	Cholera	.....	.....	1893, N. Y. Quarantine
				.....	.....	1893, U. S. Quarantine
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